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## Novel Methods for Producing Cellulose Nanocrystals from Lignocellulosic Materials and Cellulose Nanocrystals Reinforced Polymer Nanocomposites

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**Novel Methods for Producing Cellulose Nanocrystals from Lignocellulosic Materials and  
Cellulose Nanocrystals Reinforced Polymer Nanocomposites**

**Paul Busumafi Filson, M.Phil**

**Dissertation submitted to the  
Davis College of Agriculture, Forestry and Consumer Sciences  
at West Virginia University  
in partial fulfillment of the requirements  
for the degree of**

**Doctor of Philosophy  
in  
Forest Resources Science**

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**Division of Forestry and Natural Resources**

**Morgantown, West Virginia  
2008**

**Keywords: Cellulose nanocrystals, recycled pulp, hardwood and pine dissolving pulps,  
nanocomposites**

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## **ABSTRACT**

### **Novel Methods for Producing Cellulose Nanocrystals from Lignocellulosic Materials and Cellulose Nanocrystals Reinforced Polymer Nanocomposites**

Paul B. Filson

Cellulose nanocrystals (nanofibers) represent a new emerging biological source of reinforcing biofillers. In this dissertation, we report the results of a study to produce cellulose nanocrystals from recycled pulp, hardwood and pine dissolving pulps using maleic acid, ultrasonic-assisted (sono-chemical treatment) and enzyme-mediated hydrolysis followed by fragmentation of cellulose crystallites using ultrasonic treatment. Additionally, the effect of two modes of heating: conventional and microwave, on enzyme-mediated and maleic acid hydrolysis were investigated. Cellulose nanocrystals yields from maleic acid hydrolysis of lignocellulosic materials were lower than that obtained from endoglucanase mediated hydrolysis of lignocellulosic materials. Sono-chemical treatment of lignocellulosic materials produced both spherical and cylindrical cellulose nanocrystals. Yields of cellulose nanocrystals obtained from some enzyme-mediated hydrolysis treatments of lignocellulosic materials were circa 50% based on the initial weight of lignocellulosic materials. Analysis of hydrolyzates enzyme-mediated hydrolysis of pulps using high-performance liquid chromatography coupled to evaporative light scattering detection analysis showed significant amount of glucose and cellobiose. Cellulose nanocrystals were characterized by a number of physical methods including light scattering, polarizing and electron microscopy and X-ray diffraction. Cellulose nanocrystals produced were incorporated into polyimide to form nanocomposites at 0, 5, 10 and 20 wt % loadings of cellulose nanocrystals. Modulus of elasticity and tensile strength of cellulose nanocrystals reinforced polyimide nanocomposites decreased with increasing loadings of cellulose nanocrystals. Thermal analyses of the nanocomposites were further carried out. Fourier transform-infrared coupled to attenuated total reflectance disc spectra of the nanocomposites revealed interaction between hydroxyl groups in cellulose nanocrystals and carbonyl groups in polyimide.

## DEDICATION

*To my late father, Andrew Amouku Filson and my mother Elizabeth Awotwe Filson for their vision, encouragement and confidence in me. Their support has always been unwavering and unconditional.*

*Paapa, I have pursued and accomplished what you wanted me to do.*

*To my loving wife Jemima Nardu Addico-Filson for her love, patience and encouragement throughout these tough times when I was away from home working on this project.*

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This work would not have been successful without the good people I worked with in Division of Forestry and Natural Resources. I would first acknowledge the Director of Division of Forestry and Natural Resources, Dr. Joseph McNeel, for his support throughout the course of this work, especially when we needed extra financial assistance to complete this investigation. I would like to mention Dr. Jingxin Wang for his invaluable advice during the very difficult moments when the success of the project appeared a distant possibility. Due to lack of space, I could not mention all the names of the caring and lovely people in the Division, especially those in Appalachian Hardwood Center and the Support Staff in the General Office. Also, I acknowledge the cordial relationship I enjoyed with my group members, especially Oluwatosin Adedipe, Phillip George Bibu, Charlie Collins II, Medley Clay and Kofi Nkansah Jnr. throughout the course this work.

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## LIST OF ABBREVIATIONS AND SYMBOLS

<sup>13</sup> C-NMR	carbon-13 nuclear magnetic resonance
2θ	Bragg's angle of diffraction
ANL	Argonne National Laboratory
ASTM	American Standard Testing and Materials
ATR	attenuated total reflectance
CN-PI	cellulose nanocrystals reinforced polyimide nanocomposites
CP/MAS	cross polarization magic angle
CrI	crystallinity index
DMAc	N,N-dimethylacetamide
EGU	endoglucanase units
ELS	evaporated light scattering
FTIR	Fourier transform infrared
HP	hardwood dissolving pulp
HPLC	high-performance liquid chromatography
IR	infrared
M	middle lamella
MOE	modulus of elasticity
P	primary wall
PNC	polymer nanocomposite
PP	pine dissolving pulp
RP	recycled pulp
S1	outer layer of secondary wall
S2	middle layer of secondary wall
S3	inner layer of secondary wall
SEM	scanning electron microscopy
TEM	transmission electron microscopy
W	warty layer
WAXD	wide angle X-ray diffraction
XRD	X-ray diffractometry

# CHAPTER ONE

## 1. Introduction

One of the foremost promising avenues of technology development for the 21<sup>st</sup> century is the multidisciplinary science of nanotechnology. Nanotechnology is the application of molecules and structures with at least one dimension roughly between 1 and 100 nanometers (Ratner and Ratner 2003). A nanometer is one billionth of a meter – 10,000 times finer than a human hair. Potentially, nanotechnology has been recognized as a novel way of enhancing the properties of polymers through the mixing/blending of materials at this scale - nanocomposites (polymer nanocomposites, PNCs). In the manufacture of PNCs, nanoscale particles are used as fillers to reinforce a polymer matrix. Two major types of PNCs are recognized – organic and inorganic PNCs. Hybrids of the two types also exist. Typically, wood composites are macroscale composite structures consisting of approximately 90% by weight wood elements such as strands, fibers, etc. bonded together by polymer matrix, resin (10% by weight). The interests from both industrial and academic areas into PNCs stem from the observed improvements in their mechanical (strength), physical flame retardancy, gas barrier, and thermal properties over conventional composites. The enhanced performance of PNCs is attributed to their increased surface area to volume ratio.

A market research firm, Business Communications Co., Inc, (Norwalk, Connecticut) placed the world market for PNCs at 24.5 million pounds in 2003 with a value of \$90.8 million. Average annual growth rate was projected at 18.4% to \$211.1 million by 2008. Today, the leading industrial and research nano-scale fillers are layered silicate nanoclays, nano-talcs, carbon nanotubes and graphite. However, the two top industrial nanofillers are nanoclays and nanotubes. The auto industries in Detroit have used nanoclay-based PNCs in vehicles' such as the 2005 General Motors Hummer sports utility vehicles box-rail protector, sail panel and center bridge. Recently, studies in France (Favier *et al.* 1995; Anglés and Dufresne 2000) have identified the potential of producing organic nanoparticles from cellulose derived from fish and tunicates. However, a greater source of cellulose is lignocellulose found in wood. Lignocellulosic materials are renewable in nature, low cost, low density and have high specific strength and modulus. These attributes have for long led to their use as major raw materials for

the production of macroscale composites such as plywood, Parrallam<sup>R</sup>, oriented strand board, fiberboard, etc. The demonstration by Favier *et al.* 1(995); Anglés and Dufresne (2000) for the production of cellulose nanocrystals from tunicates has generated great interest in their production from lignocellulosic materials since they are abundant and renewable.

Nanotechnology operates at the thousandths of micrometer level and exploits properties of materials associated with their unique molecular properties at this scale. A nanometer is a millionth of a millimeter (human hair is 10,000-50,000 nm; diameter of human red blood corpuscles is 5,000 nm). At the nanoscale, new phenomena kick in. A case in point is Ohms law is non-operational, i.e. there is no resistance to the flow of electric current; gold assumes different colors such as blue, red, etc (Ratner and Ratner 2003). There are two main approaches in Nanotechnology: “Bottom-up” and “Top-down” approach. In “Top-down” approach, materials are manufactured by deconstruction through removal from a larger material (e.g. lignocellulose biomass) to produce a material with at least one dimension lower than 100 nanometers (cellulose nanocrystal) (Ratner and Ratner 2003) . In “Bottom-up” protocols, materials are assembled molecule by molecule (e.g. nanocoating). Nanocoating can be used to modify the surface chemical and/or physical properties of materials.

## **CHAPTER TWO**

### **2. OBJECTIVES AND DISSERTATION STRUCTURE**

#### **2.1. Objectives**

The general objectives of the study proposed here are three-fold:

- (1) To produce cellulose nanocrystals from recycled pulp, pine and hardwood dissolving pulps,
- (2) To incorporate the cellulose nanocrystals into polyimide to form cellulose nanocrystals reinforced polymer nanocomposites and finally
- (3) To characterize the resulting polymer nanocomposites (PNC).

The specific objectives of the study are listed below as:

1. Produce and characterize cellulose nanocrystals from recycled pulp, pine and hardwood dissolving pulps using an organic acid, maleic acid.
2. Produce and characterize cellulose nanocrystals from recycled pulp, pine and hardwood dissolving pulps mediated with endoglucanase enzyme.
3. Prepare and characterize cellulose nanocrystals reinforced polyimide nanocomposites from the various pulps.
4. Evaluate the mechanical properties including modulus of elasticity (MOE) and tensile strength of the various cellulose nanocrystals reinforced polyimide nanocomposites.
5. Evaluate the thermal properties of the various cellulose nanocrystals reinforced polyimide nanocomposites.

#### **2.2. Structure of the dissertation**

This dissertation consists of chapters with references to articles for publications that have been appended in the latter part of this section. Chapter 3 contains the literature review on cellulose, cellulose nanocrystals and methods of preparing and treatment of cellulose nanocrystals and cellulose respectively. Chapter 4 describes the materials and methods for the preparation and characterization of cellulose nanocrystals and cellulose nanocrystals reinforced polyimide nanocomposites. Chapter 5 presents the results and discussion of the entire study giving an

overview of the manuscripts of the various articles for publications. Chapter 6 is the conclusion and perspective for future research on this work and finally the summary of the entire work. Below are appendices that contain articles that have been both accepted and under review by various peer-review journals for publication.

Appendix A is Paper I: Filson P. B. and Dawson-Andoh B. E., “Sono-chemical preparation of cellulose nanocrystals from lignocelluloses derived materials”. *Bioresource Technology* 2008 (Accepted for publication)

Appendix B is Paper II: Filson P. B. and Dawson-Andoh B. E., “Sugar analysis of hydrolyzates from endoglucanase mediated hydrolysis of pulp using high performance liquid chromatography-evaporated light scattering detection”. *Bioresource Technology* 2008 (Under review)

Appendix C is Paper III: Filson P. B., Dawson-Andoh B. E. and Schwegler-Berry D., “Enzymatic-mediated production of cellulose nanocrystals from recycled pulp”. *Cellulose* 2008 (Under review).

Below are some of the results in the above publications that have been presented in national conferences.

1. Filson P. B. and Dawson-Andoh B. E., “Enzymatic-assisted production of cellulose nanocrystals from lignocelluloses materials”, 39th Central Regional American Chemical Society Meeting, Covington, Ky, May 20-23, 2007.
2. Filson P. B., Benjamin E. Dawson, Diane Schwegler-Berry and William E. Wallace, “Enzymatic-assisted production of cellulose nanocrystals from lignocelluloses materials”, 2<sup>nd</sup> Science Technology Research (STaR), Morgantown, West Virginia, September 17-18, 2007

## **CHAPTER THREE**

### **3. Background of study**

#### **3.1. Nanotechnology and forest products**

Nanotechnology is the single largest technological development in the last two decades and offers unique or enhanced functionality in areas of forest products such as composites, coatings, adhesion, weathering and biodeterioration.

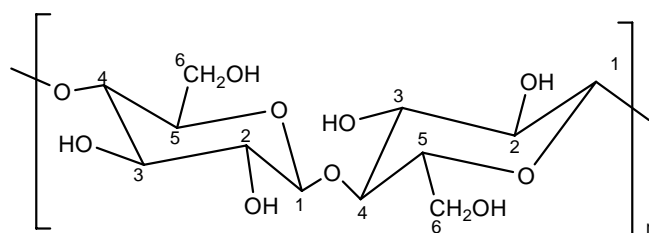
#### **3.2. Composites and fillers**

Composites consist of two main components: fillers and matrix. Simply put, fillers provide the structural strength of the material and the matrix allow the uniform transfer of stress to the fillers. The matrix ties together the fillers. Recent studies have demonstrated that strength properties, fire resistance, moisture penetration of composites can be greatly enhanced by the inclusion of small quantities (up to 5% by weight) of fillers with dimensions at the nanoscale level. Nanofillers in addition to their unique chemical properties, have outstanding physical properties notably a large surface area-to-volume ratio. Currently, the major commercial nanofiller on the market is nanoclay, a silicate derived from a non-renewable fossil source. This product has seen use in several commercial applications. It has recently been demonstrated that nanofillers can also be produced from the crystalline regions of cellulose, a major component of lignocellulose biomass. Although, the initial source of cellulose used in these studies was tunicates (marine organisms), lignocelluloses biomass has now clearly been identified as the principal potential source. Lignocellulose biomass, a renewable natural resource, is a more attractive starting material for producing nanofillers than from the current non-renewable silicate resource. Cellulose nanocrystals have strength properties (modulus of elasticity, MOE = 150 GPa) comparable to steel and some common materials (Kevlar, MOE = 156 GPa). These properties have drawn great attention from the scientific community. Also, current processes for producing cellulose nanocrystals employ environmentally incompatible (non-green) chemicals such as the use of 65% (by weight) sulfuric acid. Further, the current yield of 5% by weight makes the process a commercial non-starter. Consequently, the industry is looking for an environmentally compatible process that produces high-yield cellulose nanocrystals.



### 3.3. Cellulose: Sources and chemistry

Cellulose is the most abundant natural polymer in the biosphere. Cellulose fibers have mechanical properties which make them a promising strengthening agent in composites. It has low density and is biodegradable. In addition, cellulose is a renewable resource which has increased interest in its application as filler in the manufacture of composites (Nishino *et al.* 2004). A molecule of cellulose is composed of anhydroglucopyranose units which are joined through  $\beta$ -1,4-glycosidic bonds to form a molecular chain (Figure 1)(Fengel and Wegener 1983). The repeating units of cellulose are cellobiose, a disaccharide. The cellulose molecules (chains) are held together by hydrogen bonding. In plants, about 40 cellulose molecules are held together to form a microfibril which are deposited in the cell wall (Lewin and Goldstein 1991).



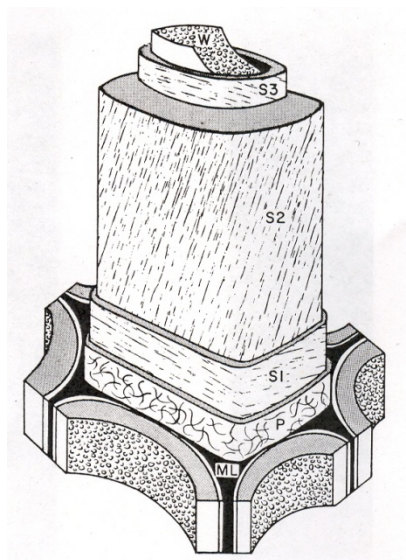
**Figure 3.1:** Chemical structure of cellulose (molecular chain)

Cellulose is distributed in all plants, from highly developed trees, to primitive organisms such as flagellates and bacteria (Fengel and Wegener 1983). It also occurs in the animal kingdom as tunicin from tunicates (Fengel and Wegener 1983). Cellulose nanocrystals are derived from a variety of natural sources. These include cotton (Dong *et al.* 1996, 1998; Heux *et al.* 2000), tunicate (Favier *et al.* 1995; Anglés and Dufresne 2000), bacteria (Araki and Kuga 2001; Grunert and Winter 2002), sugar beet (Azizi *et al.* 2004), wheat straw (Helbert *et al.* 1996) and wood (Rånby 1952; Revol *et al.* 1992; Araki *et al.* 1998).

#### 3.3.1. Chemical composition of wood and distribution of cellulose in wood cell wall

Cellulose molecules occur in wood mainly with lignin, hemicellulose, extractives and trace amounts of inorganic compounds. Cell wall consists of several layers including middle lamella (M), primary wall (P), outer layer of secondary wall (S1), middle layer of the secondary wall

(S2), inner layer of the secondary wall (S3) and warty layer (W) (Figure 3.2)( Sjostrom 1981). Cellulose molecules are distributed mostly in the secondary walls in the wood cell wall with lignin mainly in the middle lamella.



**Figure 3.2:** Simplified ultrastructure of woody cell wall showing several layers (Coté and Day1969).

### **3.3.2. Crystallinity, polymorphism and characterization of cellulose**

There are two major theories notably fringed-micelle and folded-chain lamella theories that are used in the explanation of the crystalline and amorphous behavior of materials. Fringed-micelle theory considers polymers to consist of small-sized, ordered crystalline regions referred to as crystallites imbedded in an unordered, amorphous polymer matrix (Odian 2004). Based on the fringed-micelle theory, cellulose fits perfectly into crystalline and amorphous nature of a polymer. Cellulose molecules pass through several different crystalline regions with crystallites being formed when extended. Thus, the segments from different cellulose chains are precisely aligned together and undergo crystallization. Each cellulose chain consists of ordered segments of several crystallites. Segments of the chain between the crystallites make up the unordered amorphous matrix (Figure 3.3). The percentage of cellulose in a material is also strongly dependent on the source of cellulose (Table 3.1).



**Figure 3.3:** Fringed-micelle picture of polymer (O dian 2004)

**Table 3.1:** Cellulose content of various plant materials (Fengel and Wegener 1983)

Plant material	Cellulose (%)
Cotton	95-99
Ramie	80-90
Bamboo	40-50
Wood	40-50
Bark	20-30
Mosses	25-30
Horse-tail	20-25
Bacteria	20-30

The cellulose crystallites occur in different lengths and sizes. Acid hydrolysis removes the amorphous portions leaving the crystalline portion intact, thereby increasing the crystallinity of the cellulose. Fragmentation of the cellulose crystallites leads to the formation of cellulose nanocrystals. Cellulose nanocrystals have a variety of aspect ratios (length to width ratio) depending on the source of cellulose and method of production. For instance, the aspect ratio (length-to-width) of cellulose nanocrystals from cotton is about 200 nm and 5 nm whereas that of tunicates is several microns long and 15 nm respectively (Fengel and Wegener 1983).

The high crystallinity of cellulose nanocrystals is responsible for its exceptional mechanical and physical properties which have resulted in growing interest by materials scientists. Cellulose nanocrystals have been incorporated into different polymer matrices as fillers (whiskers) in an attempt to increase the strength value of composites. Cellulose nanocrystals have MOE of 150 GPa. Modulus of elasticity relates to the stiffness of a material and it is indicative of the strength of a material. This shows the strength as well as the prospective use of cellulose nanocrystals for the development of new composites. Mechanical properties of materials are among several

critical factors that are considered in the evaluation of materials for their strength properties. However, the maximum MOE of natural cellulose is about 128 GPa which is even higher than aluminum (70 GPa) and glass fibers (76 GPa) (Page *et al.* 1971). The MOE of cellulose nanocrystals is comparable to high performance synthetic fibers including poly(p-phenylene terephthalamide) (Kevlar, Twaron) (156 GPa), Vectran (126 GPa), Technora (88 GPa) and Ekonol (130 GPa) products that are used commercially in the manufacture of composites (Nakamae *et al.* 1987).

In our attempt to understand the physical and mechanical properties of cellulose nanocrystals, the crystallinity and polymorphism of cellulose as well as the preparation of cellulose nanocrystals from a variety of sources are reviewed in the sub-sections below.

#### **3.3.4. Crystallinity and Polymorphism of Cellulose**

X-ray diffractometry (XRD), cross polarization/magic angle carbon-13 nuclear magnetic resonance (CP/MAS  $^{13}\text{C}$ -NMR), infrared (IR) and Raman spectroscopic methods have been used extensively to study the crystallinity of the different polymorphs of cellulose from different sources. (Samir *et al.* 2005). X-ray diffraction patterns of cellulose are the most widely used data to study the various phase transformations of the different polymorphs of cellulose. An X-ray diffractometer measures the various reflections of crystal planes of crystalline and amorphous materials which indicate the crystallinity of the materials. The width of X-ray diffractographs reflects the length of crystalline portion of cellulose in cellulosic materials. Crystalline materials have definitive crystal lattice structures. Cross polarization/magic angle carbon-13 nuclear magnetic resonance was used to measure the crystallinity of cellulose using different spin-lattice relaxation times of crystalline and amorphous cellulose (Horii *et al.* 1987). However, different methods had been used to determine the crystallinity indices of cellulose using X-ray diffraction data (Segal *et al.* 1959; Thygesen *et al.* 2005). Fourier transform-infrared (FT-IR) spectroscopy has also been used to study the crystallinity of cellulose I based on absorption peaks of the allomorphs (Sugiyama *et al.* 1991). Fourier transform Raman spectroscopy was recently used to determine the degree of crystallinity of cellulose I based on  $\text{CH}_2$  bending modes (Schenzel *et al.* 2005).

Cellulose exists in six different polymorphs based on their crystal lattices. These include cellulose I, II, III<sub>I</sub>, III<sub>II</sub>, IV<sub>I</sub> and IV<sub>II</sub> polymorphs (Fengel and Wegener 1983). Cellulose I and II

polymorphs are the most useful and are studied extensively because they serve as the source of the other polymorphs. Native cellulose is termed cellulose I and exists in two allomorphs, cellulose I $\alpha$  and I $\beta$  (Sugiyama *et al.* 1991). The unit crystal lattice of cellulose I $\alpha$  occurs in a triclinic state. It exists in a metastable form which changes easily into the dominant I $\beta$  form. In this crystal lattice unit, the cellulose I $\alpha$  exists as parallel cellulose chains stacked through van der Waals interactions, with progressive shear parallel to the chain axis. On the other hand, cellulose I $\beta$  exists in a monoclinic two chain unit cell, in which parallel cellulose chains are stacked with alternating shear (Wada *et al.* 2004).

Cellulose II is produced by the treatment of cellulose I with solutions of alkali or by regeneration from solution (e.g., saponification of cellulose ester) (Lewin and Goldstein 1991). However, cellulose II has high amounts of hydrogen bonding which accounts for its stability. Cellulose III is obtained by treating cellulose I or II with liquid ammonia, followed by complete evaporation of the ammonia. This leads to the production of two polymorphs of cellulose III (III<sub>I</sub> or III<sub>II</sub>) depending on the starting material (cellulose I or cellulose II). Polymorphs of cellulose IV (IV<sub>I</sub> and IV<sub>II</sub>) are obtained by heating cellulose III to 280 °C. Cellulose IV<sub>I</sub> and IV<sub>II</sub> have parallel and anti-parallel cellulose chains respectively.

### **3.4 Processing of lignocellulosic materials**

Wood can be converted to several useful products by chemical and /or physical processes. Some of these processes are: pulping, hydrothermal, acid hydrolysis, and enzymatic hydrolysis. New processes include the use of ultrasonics.

#### **3.4.1 Pulping**

Pulping is a chemical and/or mechanical process for converting wood pulp (wood fibers) by the removal and/or modification of lignin and hemicelluloses. Lignin and hemicelluloses are mostly removed by extraction with mineral acids and sodium and potassium hydroxides respectively. The amount of lignin removed depends upon the pulping process. Retention of appreciable amounts of hemicelluloses in the pulp is necessary to make strong paper. There are five major types of pulps namely bisulfate, sulfite, neutral sulfite, kraft and soda pulps (Smook 1994) based on the method of removal of lignin and hemicelluloses. About 90% of the pulps produced under

these processes are used in the production of paper. The remaining amount of pulps goes into the manufacture of non-paper pulps. Pulps may also be classified into three major categories based on their market value and purity. They include dissolving, fluff and specialty pulps (Smook 1994). Dissolving pulp is the purest form of pulp which is suitable for chemical conversion into cellulose derived products including rayon, cellophane cellulose acetate, cellulose nitrate and carboxymethyl cellulose. Fluff pulp finds its use in the absorbent medium in disposable diapers, feminine care products and hospital pads. However, specialty pulp is used in the making of filters, inner shoe soles, and laminates.

#### **3.4.2. Hydrothermal treatment of lignocellulosic materials**

Wood can be dried thermally to remove free water in wood cell walls. The effects of heat treatment on wood, especially cellulose crystallites, under oven-dried and highly moist conditions contribute immensely towards its crystallinity. The process of heating cellulosic materials under highly moist condition is referred to as hydrothermal treatment. Hydrothermal treatment significantly increases the crystallinity of cellulose as it occurs in wood and such treatment of some wood cellulose doubles the crystallinity (Bhuiyan *et al.* 2000).

#### **3.4.3. Enzymatic degradation of lignocellulosic materials**

The complex structure of cellulose requires several different enzymes for its complete degradation. Enzymes involved in the degradation of cellulose include endoglucanases, two or more cellobiohydrolases and at least one  $\beta$ -glucosidase. These cellulases act in synergy in the hydrolysis of crystalline cellulose. Endoglucanases randomly attack the amorphous regions thereby reducing the cellulose chain length (Chanzy and Henrissat 1985; Amano and Kanda 2002). However, cellobiohydrolases cleave cellobiose units either from the reducing or non-reducing ends of cellulose chains (Chanzy and Henrissat 1985; Amano and Kanda 2002). Endoglucanase enzyme was used in this study to selectively hydrolyze the amorphous regions of cellulose. The resulting cellulose crystallites were fragmented into nanocrystals using mechanical means.

#### **3.4.4. Acid hydrolysis of lignocellulosic materials**

Acid hydrolysis of cellulose has been the only main method of producing cellulose nanocrystals. Acid hydrolysis of lignocellulosics employs two forms of acids namely organic (Mosier *et al.*

2001) or mineral (Revol *et al.* 1992) acids to breakdown the lignocelluloses into sugars and cellulose particles. During acid hydrolysis, the amorphous portion of the cellulose is first degraded followed by the crystalline part (Yu and Atalla 1998). The end-products of acid hydrolysis depend on the condition of the reaction. Under mild conditions of acid and temperature for a short period of time, cellulose does not undergo total degradation into sugars but partially degrade into the crystalline state.

#### **3.4.5. Ultrasonication of materials**

Ultrasound pertains to frequencies that are above human hearing (approximately 18 kHz) (Suslick 1988). Ultrasonication or sonication uses high sound frequencies to achieve a desirable physical, chemical and mechanical change of materials. The application of ultrasound occurs in both solids and liquids (Suslick 1988). In liquids, ultrasonication occurs by the generation of millions of bubbles when they collectively collapse generates heat sometimes over 5000 K. The generation of heat is what it is used in the medium to achieve several processes including cleaning, cell disruption, fuel atomization, extraction from plants and sterilization (Suslick 1988). This unique area of chemistry where high pitch sound is employed to achieve a chemical reaction is referred to as sonochemistry. In this study, sonication is applied to cellulose particles to disrupt cellulose nanocrystals from larger cellulose particles during isolation of cellulose nanocrystals.

#### **3.5. Preparation, application and characterization of cellulose nanocrystals**

Several authors have reported on the preparation of cellulose nanocrystals from different sources (starting materials) as previously reported. The initial acid hydrolysis step removes preferentially the amorphous region leaving the crystalline region. Cellulose nanocrystals formed are subjected to centrifugation, dialysis and sonication. Centrifugation is applied to the suspension of the cellulose particles in order for the larger particles to settle at the bottom of centrifuge tubes. This is done so as to allow the supernatant liquid to be decanted. Centrifugation is repeated with water several times to wash off the hydrolyzing solution until a turbid solution is formed. The turbid solution is an indication of cellulose nanocrystals. Dialysis is employed to remove excess hydrolyzing ions from cellulose nanocrystal suspension by authors. These processes jointly lead to the disruption as well as the prevention of aggregation of the particles due to their minute

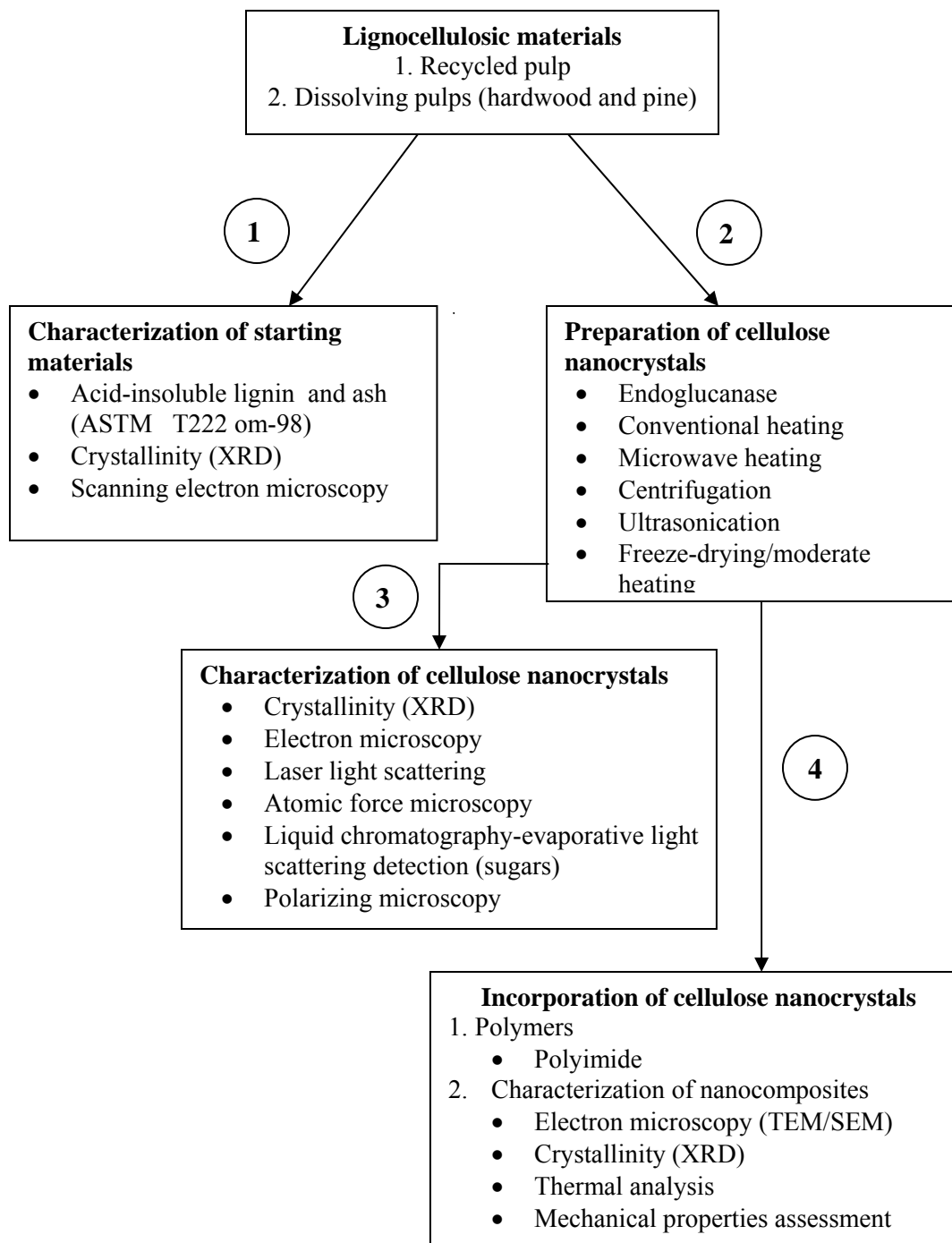
sizes. The method of preparation of cellulose nanocrystals may differ from one author to another depending on the intended use of the cellulose nanocrystals.

Cellulose nanocrystals are incorporated into different matrices at different loading levels to enhance the strength properties of the composite (Grunert and Winter 2002; Ljungberg *et al.* 2005; Kvien *et al.* 2005). Grunert and Winter (2002) surface-modified cellulose nanocrystals to reduce the hydrophilic nature to enhance their compatibility with hydrophobic thermoplastic matrix. Interestingly, there was no improvement of the mechanical properties but the thermal properties of the composite increased significantly. However, Ljungberg *et al.* (2005) prepared nanocomposite films using atactic polypropylene as the matrix and three different loadings of cellulose nanocrystals. The mechanical properties above the glass-rubber transition of the composite were drastically enhanced.

However, the yields of cellulose nanocrystals from all sources using the above processes were low (less than 20% by weight) (Edgar and Gray 2003). Also, it is reported that there is a low compatibility of cellulose nanocrystals with most matrices used in the composites that were prepared (Grunert and Winter 2002; Ljungberg *et al.* 2005; Kvien *et al.* 2005). The above mentioned issues of low yield, low compatibility between cellulose nanocrystals and polymer matrices as well as the harsh method of preparation present us with a challenge that is translated into an opportunity represented by in this study by our objectives.



**Scheme 1: Cellulose nanocrystals from lignocellulosic materials and cellulose nanocrystals reinforced polymer nanocomposites**



## CHAPTER FOUR

### 4. Materials and methods

The summary of the methods of preparation of cellulose nanocrystals and cellulose nanocrystals reinforced polyimide nanocomposites are illustrated in Scheme I above.

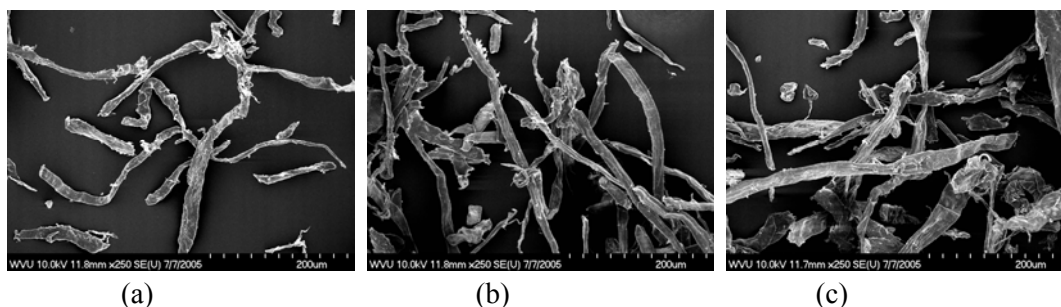
#### 4.1. Characterization of lignocellulosic materials

Recycled pulp was provided by American Fiber Resources (Fairmont, West Virginia). Recycled pulp boards were cut into pieces (approximately  $25 \times 25$  mm) and milled in a Wiley mill fitted with a 120-mesh screen. This was used as is.

Slurry of pine and hardwood dissolving pulps were provided by MeadWestvaco Corporation (Luke, Maryland). Specimen of the slurry of both pine and hardwood dissolving pulps were separately put in different beakers and placed in an oven and dried at  $103^{\circ}\text{C}$  for 24 hours to remove water. The resulting pine and dissolving pulps were later milled in a Wiley mill fitted with a 120-mesh screen. The resulting forms of the pine and hardwood pulps were used as they are throughout the experiment.

These lignocellulosic materials were analyzed for acid-soluble lignin using ASTM T222 om-98 (Table 2).

Scanning electron microscopy (SEM) characterization of recycled pulp, and hardwood and pine dissolving pulps (Figure 3) was carried out by first vacuum-drying each specimen of lignocellulosic materials for 24 hours. They were later pressed onto a double-sided tape adhered to sample holder surface and sputtered with gold. Imaging of each sample was done using Hitachi S-4700 scanning electron microscope with dispersive spectrometer. The applied accelerating voltage and current were 10 kV and  $10\ \mu\text{A}$  respectively.



**Figure 4.1:** Scanning electron microscopic images of recycled pulp (a), hardwood (b) and pine (c) dissolving pulps.

Wiley-milled recycled pulp, and hardwood and pine dissolving pulp specimens were pressed onto cylindrical sample holders 3 cm (diameter) and 4 mm (thickness) using hydraulic press at about 10,000 psi. The sample holders with compressed Wiley-milled recycled pulp were loaded onto a Panalytical X'Pert Pro Instrument fitted with an X'Celerator detector system to record XRD tracings from 5 to 50° 2 $\theta$ .

Crystallinity indices (CrI) of the starting materials were determined using Segal's Method (Segal *et al.* 1959) from the relationship below:

$$\text{CrI} = (I_{\text{crystalline}} - I_{\text{amorphous}} / I_{\text{crystalline}}) \times 100 \%$$

where,  $I_{\text{crystalline}}$  = intensity at 22.8° and

$I_{\text{amorphous}}$  = intensity at 18 to 19°

**Table 4.1:** Percentage acid-insoluble lignin and ash in lignocellulosic materials

Pulp	Mean acid-insoluble lignin (%)	Mean acid-insoluble ash (%)
Recycled	1.4 $\pm$ 0.5	0.004 $\pm$ 0.002
Hardwood Dissolving	0.11 $\pm$ 0.04	0.011 $\pm$ 0.009
Pine dissolving	0.09 $\pm$ 0.06	0.001 $\pm$ 0.000

#### 4.2. Hydrolytic enzyme (Endoglucanases)

Endoglucanase enzyme, Celluclast 1.5 L FG, was provided by Novozyme North America, Incorporated. (Franklinton, North Carolina). It had a density of 1.20 g/ml with a declared activity of 700 EGU/g. It was used as is.

#### 4.3. Polymer matrix

Polyimide resin from Alfa Aesar, A Johnson Matthey Company (Ward Hill, Massachusetts) was used as it was received. The molecular weight of polyimide used was 50,000 g/mol.

#### 4.4. Heating methods

Three different methods of heating: (1) conventional heating (Hybridization Incubator Combi-V12, FINEPCR, Yang-Chun, South Korea), (2) microwave (MARS Xpress, CEM Corporation, Matthews, North Carolina) and (3) ultrasonicator probe (Vibra-Cell VCF1500, Sonics and Materials, Inc., Newton, Connecticut) at specific power output level for a given times as specified in Table 4.2. Microwave heating was carried out first by programming the system to

ramp to the desired temperature for 10 minutes before heating the system at the attained set temperature for the set period of time. Another sonicator, Branson 2510 water-bath was also used to sonicate suspensions of cellulose particles below 20 °C in order to fragment the unhydrolyzed cellulose crystallites into cellulose nanocrystals.

#### **4.5. Design of experiment**

Cellulose nanocrystals were prepared from recycled, pine and hardwood dissolving pulps using different forms of heating. Factors studied in this study included temperature of the heating system, extent of heating of the mixture, power output of the ultrasonicator and enzyme/organic acid concentration. Response factors considered were: (1) flow birefringence, (2) appearance of turbidity of the suspension (3) shape of cellulose nanocrystals and (4) yield. The anticipated number of treatments from full factorial design was large. Consequently, a fractional factorial design of the four factors mentioned above was nested with two different concentrations of both endoglucanase enzyme and maleic acid to reduce the number of treatments. This was an exploratory design and therefore was one replicate per treatment and therefore no statistical analysis performed. After review of literature (Venkatesh *et al.* 2004; Rahkamo *et al.* 1997), conventional and microwave heating temperatures of the reaction media of 50°C (lower limit), 60°C (upper limit) and 55°C (center point) for reactions times of 40 minutes (lower limit), 50 minutes (upper limit) and 45 minutes (center-point) respectively were selected (Table 4.2). Power outputs of ultrasonicator for heating of the reaction media were carried out at 60% (lower limit), 90% (upper limit) and 75% (center-point) (Table 4.2). Different concentrations of endoglucanase enzyme (1 ml/ 2 g and 1ml/g of pulp) and maleic acids (50 ml of 50 and 200 mM) per 400 mg of pulp at two levels were added to each treatment separately. Consequently, the treatments altogether added up to 288 for both the endoglucanase and maleic acid mediated hydrolysis in the experiment.

**Table 4.2:** Heating schedules of reaction media for hydrolysis of the various lignocellulosic materials.

Heating methods	Temperature (°C)	Time (minutes)	Power output (%)
Conventional	50	60	-
	50	45	-
	60	60	-
	60	45	-
	55	52	-
Microwave	50	60	-
	50	45	-
	60	60	-
	60	45	-
	55	52	-
Ultrasonic	-	40	90
	-	30	60
	-	40	60
	-	30	90
	-	35	75

#### 4.6. Production of cellulose nanocrystals

##### 4.6.1. Production of cellulose nanocrystals from recycled pulp, pine and hardwood dissolving pulps using endoglucanase enzyme

The first step was to mix 25 ml deionized water and phosphate buffer separately with 200 mg of each pulp. The various suspensions were stirred with a magnetic stirrer and allowed to stand for 2 hours to enable the deionized water to soften the pulp. Each resulting suspension was further mixed with 100 µl of endoglucanase enzyme. Then, each suspension was placed in 75 ml vessels in the MARS Xpress microwave and 80 ml vessels in the hybridization incubator. The temperature of each suspension was first ramped to the set temperature for 10 minutes before further heating for the full length of time specified in Table 4.2. In the repeated experiments, the same mass of each pulp was treated in the same manner but 200 µl of endoglucanase enzyme was added. A control experiment for each treatment was carried out using deionized water only as hydrolyzing agent. Immediately after heating, 25 ml of ethanol was added to each treatment and stirred effectively to terminate the hydrolytic action of the endoglucanase enzyme. The resulting suspension was centrifuged at 12,000 rpm at 10°C for 10 minutes using a Sorvall RP-5B refrigerated superspeed centrifuge (DuPont Instruments, Chadds Ford, Pennsylvania) and the supernate was decanted. This process of washing the enzyme off the cellulose particles was repeated until the supernate turned turbid. The turbidity was an indication of the presence and release of the cellulose nanocrystals. The cellulose nanocrystals were later isolated. The turbid

solutions were collected after 50 ml deionized water was added and sonicated for 2 minutes. Each suspension is allowed to stand for 30 minutes. The top turbid layer is decanted. This process was repeated several times until there was no indication of turbidity in each suspension. The collected turbid solution filtered through sintered glass filter and evaporated at 50 °C in an oven until the volume of the suspension is reduced to about 25% of initial volume. The remaining suspension is freeze-dried and the cellulose nanocrystals vacuum dried to a constant dry mass. The above method of preparation of cellulose nanocrystals has been reported in manuscripts of paper III.

#### **4.6.2. Production of cellulose nanocrystals from recycled pulp, pine and hardwood dissolving pulps using maleic acid**

Fifty milliliters of maleic acid (50 mM) solution was mixed separately with 200 mg each of pulp: recycled pulp or pine dissolving pulp or hardwood dissolving pulp. Each treatment was effectively stirred using a magnetic stirrer. The heating of each suspension was ramped for 10 minutes to the target temperature (Table 4.2) and this temperature was maintained for the specified length of time using ultrasonic, microwave and conventional heating. After heating, each treatment was sonicated in a water-bath sonicator maintained below 20 °C for 15 minutes. Finally, each treatment was centrifuged at 12,000 rpm at 10°C for 10 minutes and the supernate was decanted. The maleic acid was washed off the cellulose particles several times with deionized water until a pH of 5 was obtained. A response factor of flow birefringence and turbidity respectively of supernate were determined for each treatment. The response factors are indicative of the presence of cellulose nanocrystals. Each treatment was repeated with 200 mM maleic acid solution for each pulp type. A control treatment consisted of deionized water instead of maleic acid was used.

#### **4.6.3. Liquid chromatography-evaporative light scattering detection of sugars in hydrolyzates of endoglucanase mediated hydrolysis of pulps.**

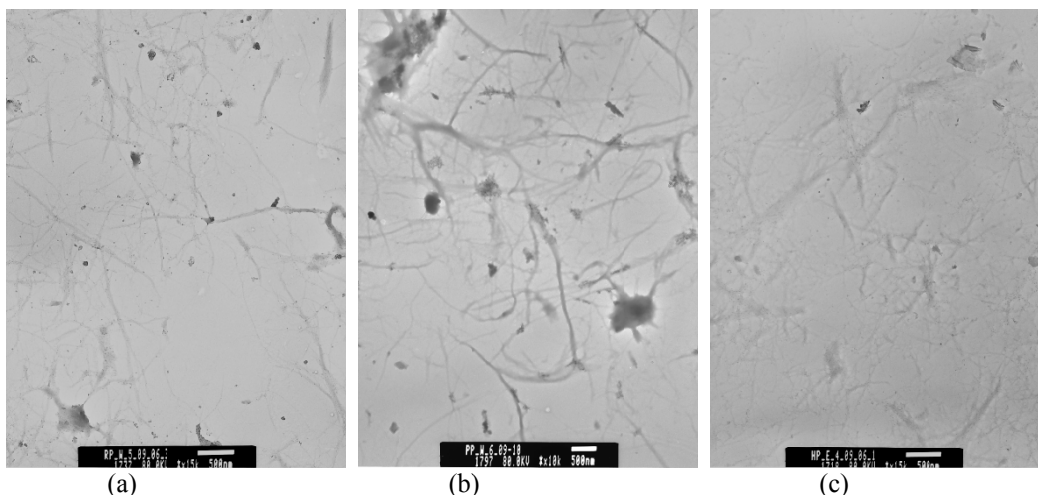
The hydrolyzates of the endoglucanase enzyme mediated hydrolysis of the various pulp types were analyzed for monosaccharides and cellobiose using liquid chromatography-evaporative light scattering detection and the results were reported in Paper II.

#### **4.7. Preparation of cellulose nanocrystals reinforced polyimide nanocomposites**

Cellulose nanocrystals from recycled pulp, pine and hardwood dissolving pulps were used to prepare cellulose nanocrystals reinforced polyimide nanocomposites. Cellulose nanocrystals loadings ranged from 0 to 20 wt% (0, 5, 10 and 20 wt %). The nanocrystals were initially dispersed in 25 ml of dimethylacetamide (DMAc) by sonicating the mixture in sonicator water-bath for 15 minutes according to Viet *et al.* (2007) or by using a magnetic stirrer. The two resulting mixtures were later added together and stirred continuously at 70 °C for 6 hours. Each film was formed by casting the resulting mixture in 15 cm-diameter petri dishes. The DMAc was evaporated from the mixture at 40 °C for 72 hours and evacuated at 30 psi in a Lab-line programmable vacuum oven. The films formed were removed from the petri dishes and placed in a dessicator. This aspect of my research work was carried out at the Argonne National Laboratory (ANL), Argonne, IL under the User Facilities program. A proposal entitled “*Characterization of structure and interactions in cellulose nanocrystals-reinforced polycarbonate and polyimide nanocomposites*” had earlier been submitted to ANL and received approval.

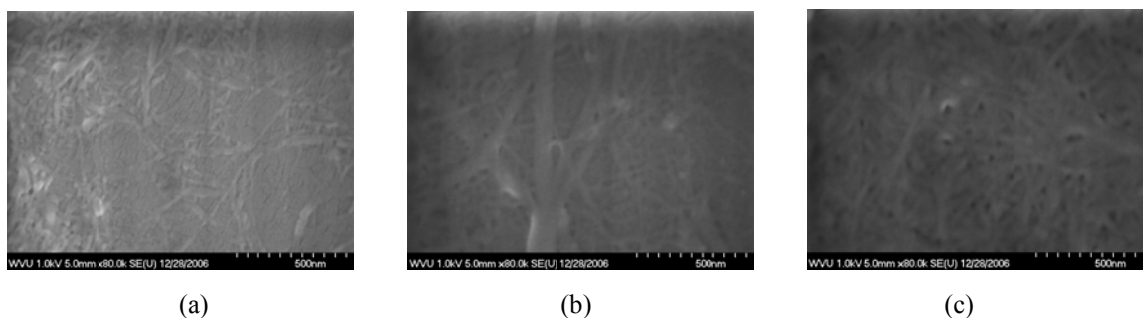
##### **4.7.1. Characterization of cellulose nanocrystals and nanocomposites**

*Electron microscopy*- A Labnet micropipette was used to draw 0.5 µl of each suspension (0.01% w/v) per treatment and loaded onto a 300-mesh carbon coated formvar copper grids (Electron Microscopy Sciences, Hatfield, Pennsylvania). Water in the suspensions on the carbon coated grids was allowed to evaporate for 24 hours. Another drop from each suspension was added onto their respective grids to increase the amount of cellulose particles and the process repeated. The grids were examined using JEOL 100CXII transmission electron microscope at 80 kV. The TEM images of the cellulose nanocrystals (Figure 4.2) were reported in Paper III.



**Figure 4.2:** TEM images of cellulose nanocrystals from (a) recycled pulp (b) hardwood dissolving and (c) pine dissolving pulps (Scale bar = 500 nm).

The cellulose nanocrystals loaded 300-mesh carbon coated formvar copper grids were also imaged using Hitachi S-4700 scanning electron microscope (SEM) with dispersive spectrometer. The applied accelerating voltage and current were 10 kV and 10  $\mu$ A, respectively. The SEM images (Figure 4.3) were taken to corroborate the TEM images.



**Figure 4.3:** SEM images of cellulose nanocrystals from (a) recycled pulp (b) hardwood dissolving pulp and (c) pine dissolving pulp (Scale bar = 500 nm).

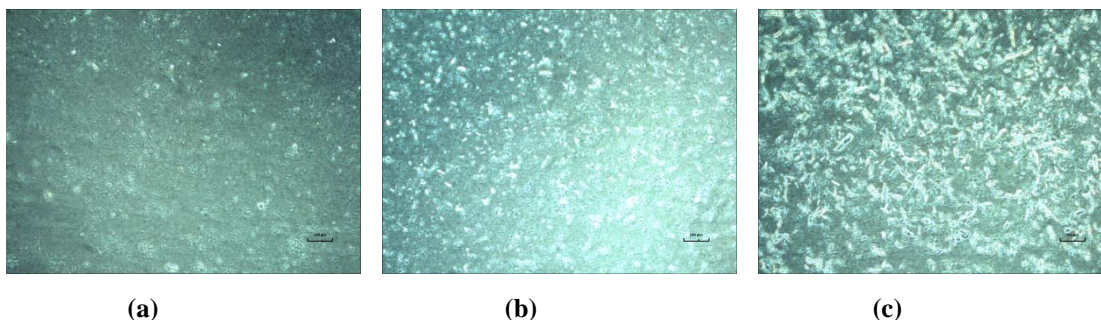
*X-ray diffraction:-* Wide angle X-ray diffraction (WAXD) patterns of cellulose nanocrystals, the various pulp types recycled, pine and hardwood dissolving pulps and their residues were recorded with a Rigaku Geiger Flex D-Max 1000 diffractometer and fiber attachment using Ni-filter Cu K $\alpha$  radiation, generated with a rotating anode generator operating at 50 kV and 120 mA. Each sample was exposed at an angle of incidence ( $\theta$ ) that varied from 10° to 50° by steps of 0.06° are reported in Paper III.

*Particle size distribution analysis:-* The particle size distribution, average molecular weight and zeta potential of colloids of cellulose nanocrystals (0.1% w/v) from different pulp sources were

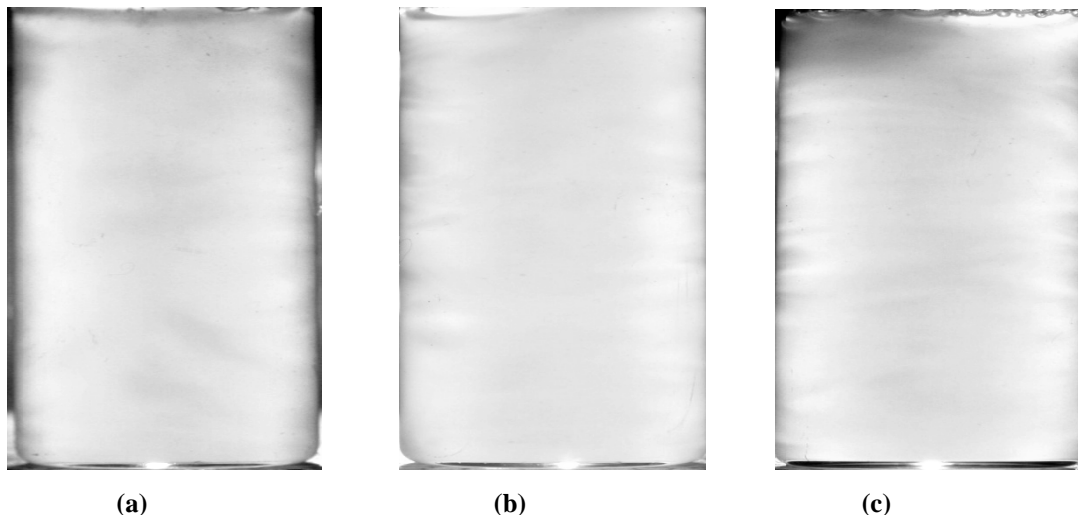


determined using Microtrac Nano Particle Size Analyzer and the results were reported in Paper III.

*Light and polarizing microscopy:-* Images of flow birefringence of (0.1% w/v) colloids of the cellulose nanocrystals between two cross polarizers were taken using an BX51 Olympus high resolution bright-field polarizing microscope (Olympus Incorporated, Melville, New York, U.S.A.) (Figure 4.4) and Canon EOS digital camera (Canon Incorporated, Japan) (Figure 4.5). These were reported in Papers III.



**Figure 4.4:** Optical images of cellulose nanocrystals obtained with a polarizing microscope at 200X (a) recycled pulp (b) hardwood dissolving pulp and (c) pine dissolving pulp



**Figure 4.5:** Flow birefringence of cellulose nanocrystals between two cross polarizing films. (a) recycled pulp (b) hardwood dissolving pulp and (c) pine dissolving pulp (Container is 20 mm in diameter).

*Birefringence of cellulose nanocrystals:-* Cellulose nanocrystals are anisotropic as result of two distinct indices of refraction formed when polarized light is incident of on them. This is characteristic of crystalline materials. This property of crystalline materials is referred to as birefringence. Birefringence of cellulose nanocrystals is observed between two cross polarizers (Figure 4.5).

*Fourier transform Infrared spectroscopy:-* Fourier transform infrared (FTIR) spectra of films of each nanocomposite were recorded in reflectance mode using Bruker Hyperion 3000 spectrometer coupled to attenuated total reflectance (ATR) objective (Bruker Optics, Ettlingen, Germany) to study the molecular groups interaction between the cellulose nanocrystals and the polyimide. Sixteen scans were acquired in the 4000-600  $\text{cm}^{-1}$  range with resolution of 4  $\text{cm}^{-1}$ .

*Thermogravimetric analysis:-* Cellulose nanocrystals reinforced polyimide nanocomposites were thermogravimetrically analyzed using Q500 (TA Instruments, New Castle, Delaware, U.S.A.) to study the rate of heat for mass-loss at 10  $^{\circ}\text{C}/\text{minute}$  of 12 - 14 mg of each sample of nanocomposites from 25  $^{\circ}\text{C}$  to 550  $^{\circ}\text{C}$  under nitrogen gas.

*Dynamic mechanical analysis:-* Assessment of mechanical properties including tensile strength and modulus of elasticity of cellulose nanocrystals reinforced polyimide nanocomposites was conducted. Strips of films of the nanocomposites were cut into approximate dimensions of  $8 \times 1.6 \times 0.18 \text{ mm}^3$  and maintained at relative humidity of 52%. Three film strips of each cellulose nanocrystals reinforced polyimide nanocomposites were tested in tension mode on Q800 (TA Instruments, New Castle, Delaware, U.S.A.) at a strain rate of  $5.00 \times 10^{-5} \text{ s}^{-1}$  at 25  $^{\circ}\text{C}$ .

## CHAPTER FIVE

### 5. Results and Discussion

#### 5.1. Overview

Cellulose nanocrystals were produced from three types of pulp: (1) recycled pulp, (2) hardwood dissolving pulp and (3) pine dissolving pulp by hydrolysis with endoglucanase enzyme and maleic acid mediated by conventional, microwave and ultrasonication forms of heating. In an earlier study, cellulose nanocrystals were produced by ultrasonic assisted maleic acid hydrolysis of recycled pulp (low crystallinity) and microcrystalline wood cellulose, Avicel (high crystallinity). No form of external heating was applied here. This process yielded spherical and cylindrical forms of cellulose nanocrystals (Paper I). Very few treatments in this study produced cellulose nanocrystals. The yields of the latter were also low (10 - 15%).

The same experimental design with two different volumes (100 and 200  $\mu$ l) of endoglucanase enzyme per 200 mg of lignocellulosic materials was used to produce cellulose nanocrystals with yields of 10-25% and 10-48% (based on the initial weight of lignocellulosic material) treatments included those with 100  $\mu$ l of endoglucanase enzyme per 200 mg of lignocellulosic materials and treatments with 200  $\mu$ l of endoglucanase per 200 mg of lignocellulosic materials, respectively. Treatment at 50 °C for 60 minutes (Paper III) yielded the highest amount of cellulose nanocrystals (48%) from the three lignocellulosic materials using microwave heating (Table 5.1). Hydrolyzates from enzymatic hydrolysis of each lignocellulosic material were later analyzed for glucose and cellobiose content using high- performance liquid chromatography (HPLC) coupled to evaporative light scattering (ELS) detector (Paper II). The concentrations of glucose and cellobiose in hydrolyzates were 110 - 250 ppm and 60 - 200 ppm respectively with some of the hydrolyzates containing undetectable amounts of mannose, xylose, arabinose, galactose, rhamnose. Cellulose nanocrystals from the pulp types were cylindrical in shape with a width of 50 - 80 nm and a length of 100nm - 1.8  $\mu$ m. Average zeta potentials of cellulose nanocrystals suspensions (0.1 % by weight) of hardwood and pine dissolving pulps were -14.01 and -22.18 mV respectively. This indicates a favorable stable suspension. However, the average zeta potential of cellulose nanocrystals in suspension from recycled pulp was -31.37 mV indicating high stability (Paper III). The average molecular weight of cellulose nanocrystals from recycled pulp, pine and hardwood dissolving pulps were  $1.65 \times 10^{11}$ ,  $1.84 \times 10^{11}$  and  $2.81 \times 10^7$  g/mol

respectively as determined by dynamic light scattering. The high average molecular weight of cellulose nanocrystals of recycled and pine dissolving pulps was ascribed to aggregation of cellulose nanocrystals.

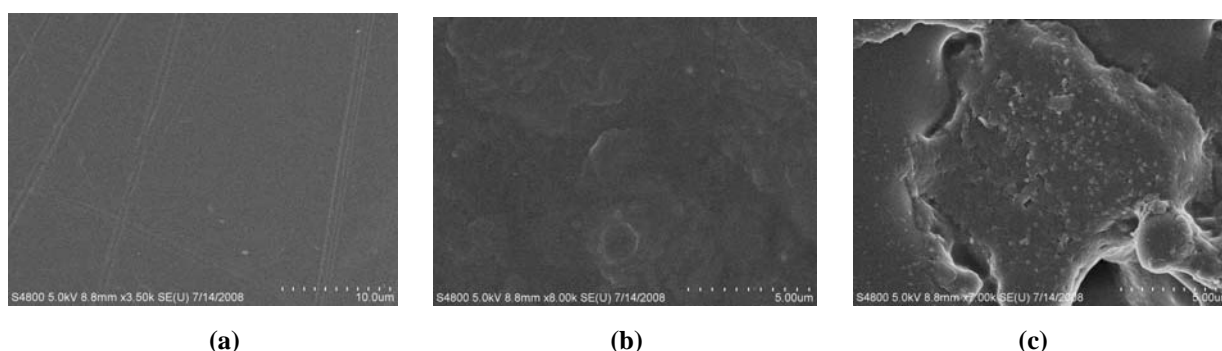
**Table 5.1:** Experimental results for properties and yield of cellulose nanocrystals from recycled pulp

Conventional heating	Factor responses			
	Characteristics of suspension	Flow birefringence	Shape of particles	Yield (%)
C-1-E	Turbid	Yes	Cylindrical	10.7
C-1-B	Clear	No	-	-
C-1-W	Turbid	Yes	Cylindrical	7.4
C-2-E	Turbid	Yes	Cylindrical	29.0
C-2-B	Turbid	Partial	Cylindrical	25.7
C-2-W	Turbid	Yes	Cylindrical	7.1
C-3-E	Turbid	Yes	Cylindrical	15.0
C-3-B	Clear	Partial	Cylindrical	21.0
C-3-W	Turbid	Yes	Cylindrical	3.7
C-4-E	Turbid	Yes	Cylindrical	3.4
C-4-B	Turbid	Partial	Cylindrical	18.0
C-4-W	Turbid	Yes	Cylindrical	1.5
CC-E	Turbid	Yes	Cylindrical	16.0
CC-B	Clear	Partial	Cylindrical	11.0
CC-W	Turbid	Yes	Cylindrical	4.9
Microwave heating				
M-1-E	Turbid	Yes	Cylindrical	34.9
M-1-B	Clear	Partial	Cylindrical	10.9
M-1-W	Turbid	Yes	Cylindrical	12.8
M-2-E	Turbid	Yes	Cylindrical	38.2
M-2-B	Turbid	Yes	Cylindrical	26.4
M-2-W	Turbid	Yes	Cylindrical	4.9
M-3-E	Turbid	Yes	Cylindrical	22.9
M-3-B	Clear	Partial	Cylindrical	15.9
M-3-W	Turbid	Yes	Cylindrical	6.2
M-4-E	Turbid	Yes	Cylindrical	20.5
M-4-B	Turbid	Yes	Cylindrical	15.0
M-4-W	Turbid	Yes	Cylindrical	6.6
MC-E	Turbid	Yes	Cylindrical	18.2
MC-B	Clear	Partial	Cylindrical	13.0
MC-W	Turbid	Yes	Cylindrical	6.0

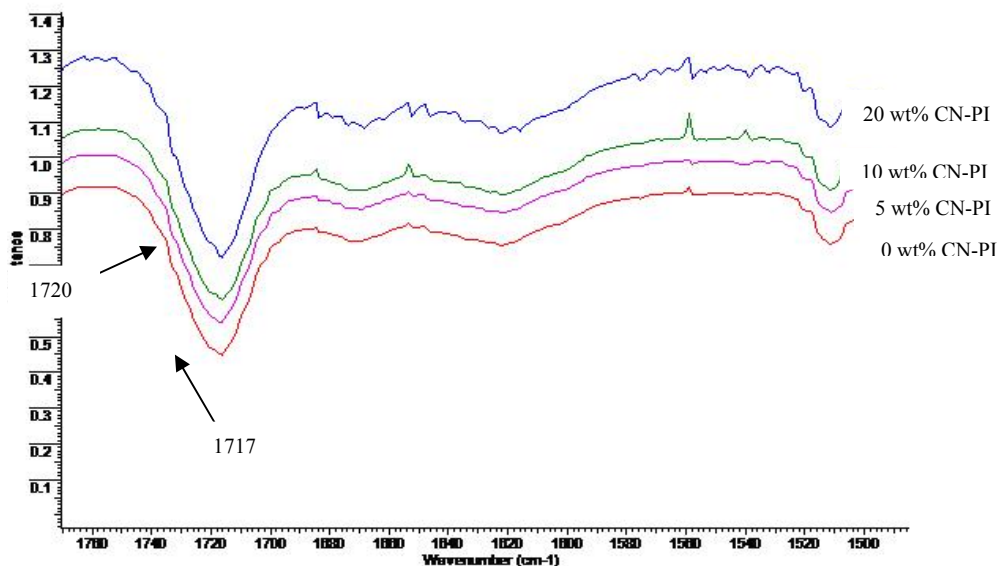
C = convectional heating; M= microwave heating; CC = conventional heating at center-point temperature; MC = microwave heating at center-point temperature; E = Enzyme in deionized water; B =Enzyme in sodium phosphate buffer and W = water only.

Cellulose nanocrystals reinforced nanocomposites were imaged using SEM and polarizing microscope to study the relationship between the polyimide matrix and the cellulose nanocrystals filler (Figure 5.1). The latter occurred as white dots in the polyimide matrix. The dots increased

with increasing loadings of cellulose nanocrystals in the nanocomposites. The molecular interactions between cellulose nanocrystals filler and the polyimide matrix studied using ATR-FTIR spectroscopy. A prominent peak was observed at  $1716\text{ cm}^{-1}$  with a weak shoulder peak at  $1720\text{ cm}^{-1}$ , ascribed to C=O stretching vibration of carbonyl groups in polyimide (Figure 5.2). As the loadings of cellulose nanocrystals increased, a weak peak at  $1720\text{ cm}^{-1}$  emerged. This peak can be attributed to the weak interactions between carbonyl groups in the polyimide and the hydroxyl groups in the cellulose nanocrystals. This further suggests that interactions between cellulose nanocrystals and polyimide molecules are weak.



**Figure 5.1:** SEM images of cellulose nanocrystals reinforced polyimide nanocomposites with loadings of (a) 0 wt % (b) 10 wt% and (c) 20 wt % cellulose nanocrystals.



**Figure 5.2:** Typical ATR-FTIR spectra of cellulose nanocrystals reinforced polyimide nanocomposites.

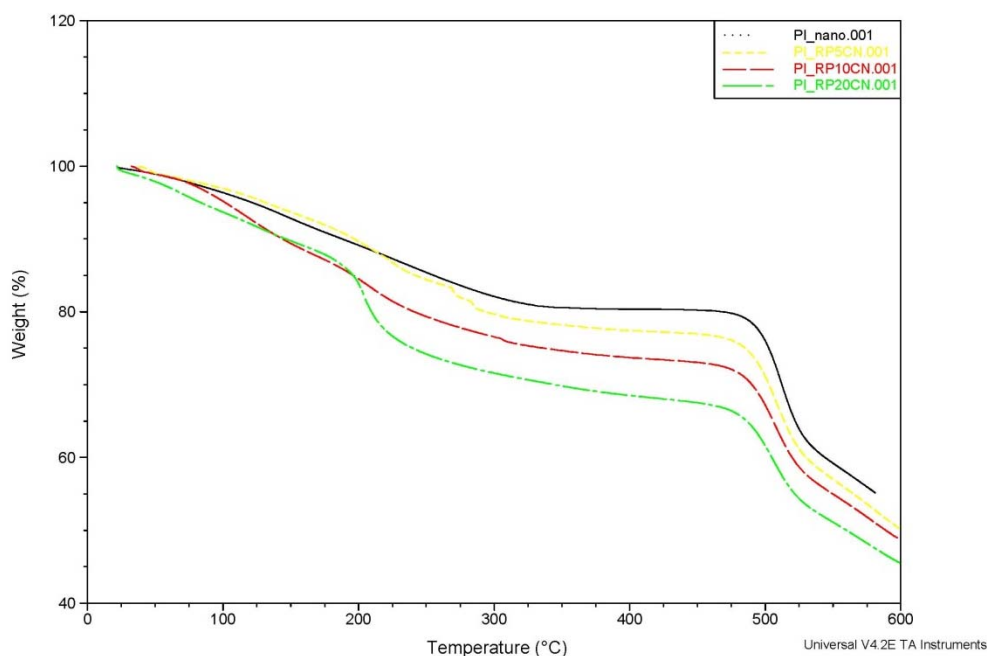
\*CN-PI – Cellulose nanocrystal reinforced polyimide nanocomposites

Young's modulus and tensile strength of the cellulose nanocrystals reinforced polyimide nanocomposites with different loadings of cellulose nanocrystals were also determined from the slope of the linear region of the stress-strain curves. Both Young's modulus and tensile strength of the nanocomposites decreased with increasing loadings of cellulose nanocrystals (Table 5.2). This is attributed to aggregation of cellulose nanocrystals as their levels increased in nanocomposites reducing van der Waal's and hydrogen bonding interactions with molecular groups in polyimide.

**Table 5.2:** Mechanical properties of polyimide and cellulose nanocrystals reinforced polyimide nanocomposites results at 25 °C.

Cellulose nanocrystal reinforced polyimide nanocomposites	Modulus of elasticity/GPa	Tensile strength/MPa
PI	$2.0 \pm 0.00$	$40.3 \pm 4.8$
5%wtRP-PI	$2.0 \pm 0.00$	$35.6 \pm 3.9$
10%wtRP-PI	$0.97 \pm 0.60$	$22.9 \pm 5.1$
20%wtRP-PI	$0.80 \pm 0.20$	$20.9 \pm 7.2$
5%wtHP-PI	$1.70 \pm 0.60$	$36.7 \pm 4.0$
10%wtHP-PI	$1.00 \pm 0.00$	$29.6 \pm 3.3$
20wt%HP-PI	$0.87 \pm 0.23$	$24.6 \pm 8.9$
5wt%PP-PI	$1.70 \pm 0.60$	$27.7 \pm 6.2$
10wt%PP-PI	$0.93 \pm 0.12$	$28.0 \pm 8.1$
20wt%PP-PI	$0.87 \pm 0.06$	$19.6 \pm 1.2$

\*RP, HP and PP are cellulose nanocrystals from recycled pulp, hardwood and pine dissolving pulps. PI- polyimide.



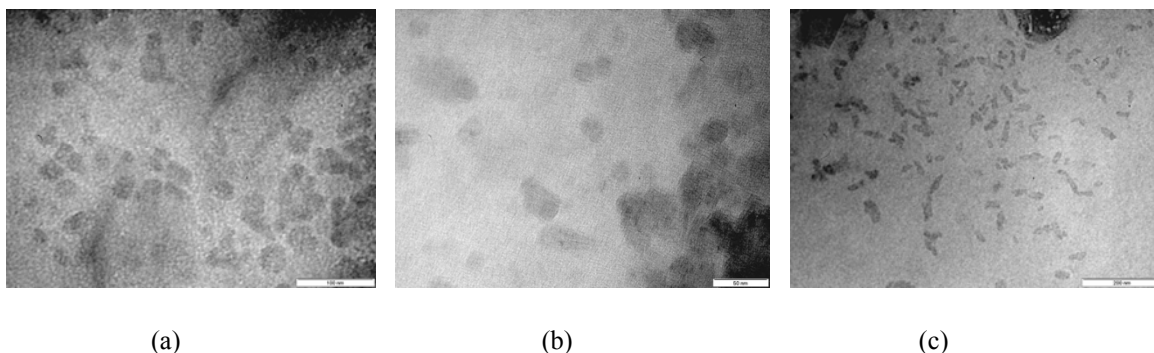
**Figure 5.3:** Thermogravimetric analysis curves under nitrogen for polyimide and cellulose nanocrystals reinforced polyimide nanocomposites.

Figure 5.3 shows that polyimide and cellulose nanocrystals reinforced polyimide nanocomposites thermally degrade under nitrogen differently as the loadings of cellulose nanocrystals increased. Polyimide thermally degraded at about 500 °C. The mass loss observed from the transformation of the thermogravimetric analysis curve between 25 to 300 °C for polyimide was attributed to the residual dimethylacetamide in the matrix. However, cellulose nanocrystals in each nanocomposite thermally degraded at about 200 °C. This was more prominent in the 20% loading of cellulose nanocrystals in nanocomposite.

### 5.1.1. Manuscripts

**Paper I:** Sono-chemical preparation of cellulose nanocrystals from lignocelluloses derived materials. *Bioresource Technology* 2008 (Accepted for publication)

Paper I examined the production of cellulose nanocrystals from microcrystalline wood cellulose, Avicel and recycled pulp of wood pulp using sono-chemical-assisted hydrolysis. Two solvent systems, deionized water and maleic acid, were evaluated. In deionized water, Avicel produced cellulose nanocrystals with average diameter of  $21 \pm 5$  nm (minimum 15 nm and maximum 32 nm) (Figure 5.4a and 5.4b). Cellulose nanocrystals from recycled pulp were not distinctively spherical but had an average diameter of  $23 \pm 4$  nm (minimum 14 nm and maximum 32 nm). Maleic acid (50 mM) hydrolysis of Avicel at 15 °C mediated with ultrasonication at 90% power output for 9 minutes produced cellulose nanocrystals which were cylindrical in shape and were of dimensions, length  $65 \pm 19$  nm and width 15 nm (Figure 5.4c). This study was a preliminary work carried out to prove the principle of production of cellulose nanocrystals using sono-chemical method.



**Figure 5.4:** Transmission electron microscopic images of cellulose nanocrystals of (a) recycled pulp (b) Avicel (c) Avicel. (Scale bar = 100 nm)

**Paper II:** Sugar analysis of hydrolyzates from endoglucanase mediated hydrolysis of pulp using high performance liquid chromatography-evaporated light scattering detection. *Bioresource Technology* 2008 (Under review)

Paper II analyzed hydrolyzates from endoglucanase mediated hydrolysis of pulps that were hydrolyzed to produce cellulose nanocrystals. High performance liquid chromatography coupled to evaporative light scattering detector was used to quantitatively determine glucose and cellobiose from hydrolyzates from the production of cellulose nanofillers using endoglucanase mediated hydrolysis of recycled pulp, pine and hardwood dissolving pulps. A model pulp hydrolyzate was constituted from range of concentrations 160-4220 ppm of glucose, mannose, xylose, rhamnose, arabinose, galactose and cellobiose. Prevail Carbohydrate ES 5 $\mu$  column was more suitable for achieving the chromatographic separation of the model pulp hydrolyzate into the various constituent sugars than YMC-Pack Polyamine II. Calibration curves for the various sugars in the mixtures were developed. Glucose and cellobiose were clearly detectable in the various pulp hydrolyzates from recycled pulp, pine and hardwood dissolving pulps comparing the retention times to the constituents of the standard model hydrolyzates. However, the amount of glucose was generally higher than cellobiose present in the pulp hydrolyzates (Table 5.3).

**Table 5.3:** Composition of pulp hydrolyzates of endoglucanase mediated hydrolysis of recycled and dissolving pulps

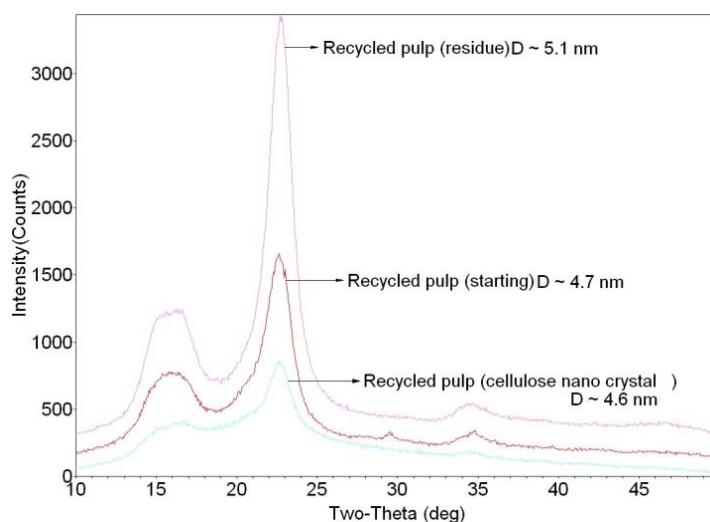
	Conventional heating			Microwave heating		
	PP-1	RP-1	HP-1	PP-2	RP-2	HP-2
Pulps						
Glucose	8.2	5.9	6.9	6.1	12.3	5.5
Cellobiose	4.6	9.9	4.9	nd	2.9	nd

**Paper III:** Enzymatic-mediated production of cellulose nanocrystals from recycled pulp. *Cellulose* 2008 (Under review)

Endoglucanase was used to hydrolyze recycled pulp to produce cellulose nanocrystals. Highest yield of cellulose nanocrystals were obtained by treatment with 1 $\mu$ l of endoglucanase per 1 mg recycled pulp produced the highest yield of cellulose nanocrystals at 50 °C for 60 minutes of microwave and conventional heating. Of the two modes of heating investigated, microwave heating at each treatment gave a higher yield than conventional heating. Transmission and scanning electron microscopic analysis of cellulose nanocrystals suspensions showed widths of



30 nm to 80 nm and lengths of 100 nm to 1.8  $\mu\text{m}$ ). This was within the cellulose crystals dimensions (100 nm to 3.5  $\mu\text{m}$ ) obtained using dynamic light scattering analysis. The average zeta potential of cellulose nanocrystals was -31.37 mV. X-ray diffraction of cellulose nanocrystals, recycled pulp and residue of recycled pulp showed gradual change in particle size (Figure 5.5).



**Figure 5.5:** X-ray diffraction patterns of cellulose nanocrystals, recycled pulp (initial) and recycled pulp (residue)

## CHAPTER SIX

### 6.1 Conclusions and perspectives on the future

This study demonstrated that the geometry of cellulose nanocrystals is dependent upon the method of production. Cellulose nanocrystals produced using sono-chemical hydrolysis of lignocellulosic materials exhibited two geometrical forms: spherical and cylindrical, depending on the experimental conditions employed. The exploratory results from the experimental design for this study also shows the potential of producing cellulose nanocrystals at high yields (circa 50% by weight of initial lignocellulosic materials) from recycled and dissolving pulps using endoglucanase enzyme mediated by microwave or conventional heating. On the basis of cellulose nanocrystal yields from recycled pulp, hardwood and pine dissolving pulps, microwave was a better heating medium than conventional heating.

Production of cellulose nanocrystals using enzymes represents a novel “green” approach that eliminates the current use of environmentally-incompatible highly concentrated mineral acids.

High-performance liquid chromatography coupled with evaporated light scattering detector analysis of endoglucanase enzyme mediated hydrolyzates of all pulp types clearly demonstrated the significant amount of by-products: glucose, cellobiose and other oligomers of glucose, available in this process as potential feedstock for the production of biofuels and other biochemicals thereby utilizing lignocellulosic materials wholly.

Cellulose nanocrystals obtained from endoglucanase enzyme-mediated hydrolysis of the three lignocellulosic materials were used as fillers in a polyimide nanocomposite at 5, 10 and 20% loadings. Evaluation of the mechanical properties of the polyimide nanocomposites revealed that modulus of elasticity and tensile strength decreased with increasing cellulose nanocrystals loadings. This could be attributed to the aggregation of cellulose nanocrystals without interacting strongly with molecules of polyimide.

Finally, future studies should examine conditions for preparing nanocomposites as well as the effect of different loadings of cellulose nanocrystals on the strength properties of the polyimide nanocomposites.

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## **APPENDIX A**

Paper I: (Accepted for publication in Bioresource Technology, 2008)

### **Title:**

**Sono-chemical preparation of cellulose nanocrystals from lignocellulose derived materials**

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### **Abstract:**

This study examined the production of cellulose nanocrystals from microcrystalline wood cellulose, Avicel and recycled pulp of wood pulp using sono-chemical-assisted hydrolysis. Two hydrolysis systems: deionized water and maleic acid were evaluated. In deionized water, Avicel produced cellulose nanocrystals with average diameter of  $21 \pm 5$  nm (minimum 15 nm and maximum 32 nm). Cellulose nanocrystals from recycled pulp were not distinctively spherical and had an average diameter of  $23 \pm 4$  nm (minimum 14 nm and maximum 32 nm). Maleic acid (50 mM) sono-chemical assisted hydrolysis of Avicel at 15 °C and 90% power output for 9 minutes produced cellulose nanocrystals which were cylindrical in shape and were of dimensions, length  $65 \pm 19$  nm and width 15 nm.

### **Keywords:**

Biomass, cellulose, cellulose nanocrystals, avicel, recycled pulp, hydrolysis, sonochemistry

**Main Text:**

## Introduction:

Fillers are incorporated into composites to enhance performances such as stiffness, dimensional stability, fire, moisture diffusion, and thermal resistance. They are usually produced from organic and inorganic materials and examples include cellulosic fibers, calcium carbonate, kaolin, aluminum hydrate, silica, talc, glass microspheres, etc. Fillers are produced primarily from non-renewable fossil sources and biomass. Since the former are finite, their continuing availability in the future is of growing concern. As a consequence, there is now an increasing need to develop new class of fillers from non-finite sources. One such promising alternative source is biomass. Biomass which is renewable and sustainable is derived from plants or animals.

Some novel technologies today are making it possible to produce materials that have hitherto been produced from fossil sources. Materials benefiting from such novel and new technologies are new biomass-derived products such as cellulose nanocrystals or whiskers. To-date, cellulose nanocrystals have been produced from diverse cellulose containing sources such as lignocelluloses biomass, wood (Rånby 1952); tunicates (Favier et al., 1995); cotton (Dong et al., 1996); wheat straw (Helbert et al., 1996), and bacteria (Araki and Kuga 2001). The wood cell wall is a bio-nanocomposite consisting primarily of three biopolymers: cellulose, hemicelluloses, and lignin. Cellulose in lignocelluloses is built from cellulose microfibrils which are bound together by lignin and hemicellulose. Cellulose nanocrystals are nanoparticles which have been shown to enhance mechanical strength performance such as stiffness (Chakraborty et al., 2005; Nishino et al., 2004; Grunert and Winter 2002; Favier et al., 1995) when used as fillers.



The biopolymer cellulose consists of glucose units linked by 1,4- $\beta$ -glycosidic bonds with cellobiose, a dimer, as its repeating unit. It is partially crystalline and amorphous (Fengel and Wegener 1983). It exists in four different polymorphic states which include crystalline cellulose I (native cellulose) and cellulose II, and amorphous cellulose III and IV. Additionally, native cellulose exists in two allomorphs which are the only crystalline structure in nature.

Cellulose nanocrystals are generally produced by a two-step process: (1) initial hydrolysis to remove the amorphous regions of the cellulose polymer, and (2) fragmentation of the crystalline segments to produce nanocrystals. These nanoparticles have received increasing attention due to their extraordinary mechanical properties such as high Young's modulus and tensile strength (Šturcova et al. 2005). So what are cellulose nanocrystals? They are cellulose crystallites with at least one dimension equal or less than 100 nm. So far, the main production method has been hydrolysis of a partially crystalline cellulose material with about 60% by weight of sulfuric acid followed by fragmentation (mechanical, sonication, etc.) of the resulting crystalline cellulose (Kvien et al., 2005; Durjardin et al., 2003; Podsiadlo et al., 2005; Beck-Candanedo et al., 2005).

The hydrolyzing agent, sulfuric acid introduces bulky ester groups onto the hydroxyl groups and stabilizing the cellulose nanocrystals in solution by preventing its agglomeration (Araki et al., 1998). However, the use of sulfuric acid has a number of important drawbacks such as potential degradation of cellulose; corrosivity, and environmental incompatibility. As alternatives to sulfuric or other mineral acids, we have examined a number of organic acids. One such organic acid is maleic acid. Maleic acid hydrolysis of cellulose gives a high yield of glucose with minimum degradation of glucose (Mosier et al., 2001). Thus, we hypothesize that under appropriate experimental conditions, maleic acid can produce higher yields of cellulose

nanocrystals. Further, the two carboxylic groups on the first and fourth carbons of this four-carbon dicarboxylic acid can be viewed as “claws” capable of forming bulky groups when esterified to the hydroxyl groups of cellulose. This may also help stabilize the resulting cellulose nanocrystals in solution.

Sonication (ultrasonication) is the application of sound energy to physical and chemical systems. In liquids, sonication produces bubbles that grows and implodes hot spots that cause acoustic cavitations, formation, growth and implosive collapse of bubbles to produce hot spots (Suslick et al., 1991). Many investigators apply sonication after acid hydrolysis of cellulose to disperse cellulose nanocrystals produced (Kvien et al., 2005; Durjardin et al., 2003; Podsiadlo et al., 2005; Beck-Candanedo et al., 2005). Some current applications of sonication include the production of nanoparticles from metals (Bellisent et al., 1993, Suslick et al., 1996; Fujimoto et al., 2001). A similar approach is adopted in this study to produce nanoparticles from cellulosic and lignocellulosic materials.

However, extensive review of the literature shows no or very little application of sono-chemical-assisted hydrolysis of cellulosic materials to the production of nanocrystals. In the present work, we investigated the sono-chemical-assisted hydrolysis of two materials: Avicel and recycled pulp, using two hydrolysis systems: deionized water and maleic acid. With deionized water, sonication was applied at 1050 W for 5 and 10 minutes respectively for both materials. Sonication using maleic acid employed a fractional factorial experimental design with the following factors: power of ultrasound; concentration of maleic acid; time of ultrasonication and temperature of water-bath. The starting materials were Avicel and recycled pulp.

## **Materials and Methods**

### **Materials**

Recycled pulp (1% lignin) was provided by American Fiber Resources (Fairmont, West Virginia, U.S.A.). Recycled pulp was produced from waste wood pulp and used business papers. Microcrystalline wood cellulose, Avicel, was purchased from Sigma Chemicals (Ohio, U.S.A.) and used as is. Maleic acid powder from Sigma-Aldrich Incorporated, (St. Louis, Missouri, U.S.A.) was purchased from Sigma-Aldrich (Ohio, USA). All water used in the study was deionized water obtained using Corning Mega-pure (distilled water) and Barnstead E-pure purification systems (deionized water).

### **Methods**

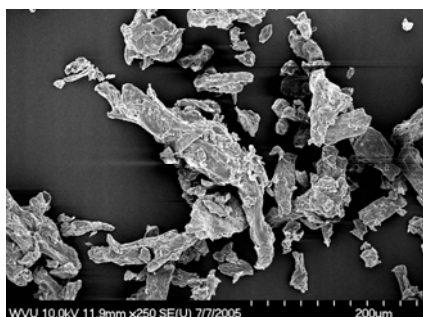
#### **Preparation of recycled pulp**

Recycled pulp boards were cut into pieces (approximately 50 X 50 mm) and milled in a Wiley mill fitted with a 40-mesh screen. The resulting material was kept in a dessicator at 50% relative humidity until use. Avicel was used as is.

#### **Characterization of Avicel and Recycled Pulp**

##### **Scanning Electron Microscopy (SEM) characterization of Avicel and recycled pulp**

Wiley-milled recycled pulp and Avicel (Figure 1) were vacuum-dried for 24 hours; pressed onto a double-sided tape adhered to sample holder surface and sputtered with gold. Imaging of each sample was done using Hitachi S-4700 scanning electron microscope with dispersive spectrometer. The applied accelerating voltage and current were 10 kV and 10  $\mu$ A, respectively.



(a) Avicel



(b) Recycled pulp

**Figure 1.** SEM pictures of untreated (a) Avicel and (b) recycled pulp

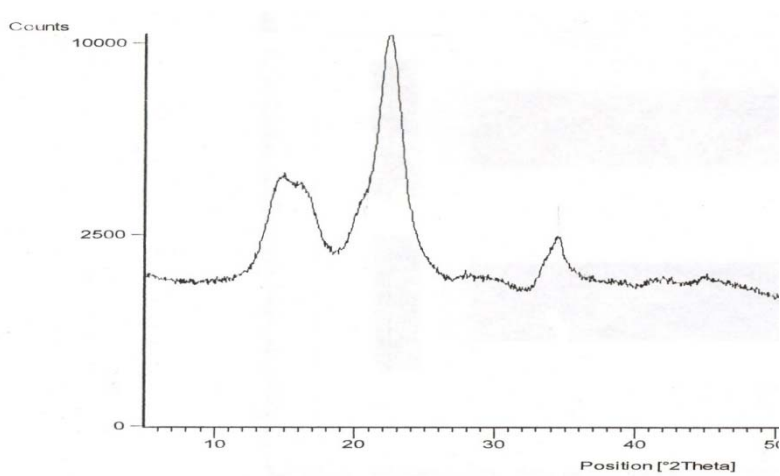
X-ray diffraction (XRD) of Avicel and recycled pulp

Wiley-milled recycled pulp and avicel samples were pressed onto cylindrical sample holders of 3 cm (diameter) and 4 mm (thickness) using hydraulic press at about 10,000 psi (Awadel-Karim et al. 1999). Residues of both recycled pulp and Avicel after hydrolysis were also analyzed in a similar manner. The sample holders with compressed Wiley-milled recycled pulp and Avicel were loaded onto a Panalytical X'Pert Pro Instrument fitted with an X'Celerator detector system to record XRD tracings from 5 to 50° 2θ with data acquisition taken at 0.04° for 1 sec.(Figure 2).

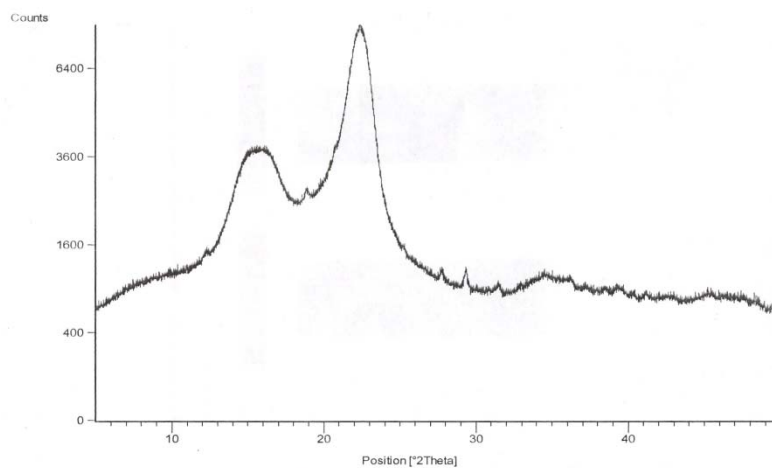
Crystallinity indices (CrI) of the starting materials were determined using Segal's Method (Segal et al. 1959) from the relationship below:

$$\text{CrI} = (I_{\text{crystalline}} - I_{\text{amorphous}} / I_{\text{crystalline}}) \times 100 \%$$

Where,  $I_{\text{crystalline}}$  = intensity at 22.8° and  $I_{\text{amorphous}}$  = intensity at 18 to 19°



(a)



(b)

**Figure 2:** Diffractograms of (a) Avicel and (b) recycled pulp residues from maleic acid hydrolysis.

#### Design of experiment – Production of Cellulose Nanocrystals

The Null hypothesis for this study was that sono-chemical-assisted deionized water or maleic acid hydrolysis of cellulosic and lignocellulosic materials does not yield cellulose nanocrystals.

Sono-chemical-assisted hydrolysis using deionized water examined only one factor: time of ultrasonication (5 and 10 minutes, Table 1). For maleic acid, a fractional factorial experimental design (Unscrambler Software, NJ) consisting of four factors with two levels of each factor was used. The factors were: maleic acid concentrations (50 and 100 mM), prepared by dissolving 11.6 and 23.2 g separately in 1 L deionised water respectively, time of ultrasonication (6 and 9 minutes), power of ultrasonicator (60 and 90% power output) and temperature (15 and 35 °C) of water-bath were considered (Table 2).

**Table 1.** Treatment conditions for sonochemical-assisted water hydrolysis of Avicel and recycled pulp

Cellulosic materials	Power Output of ultrasonicator (%)	Time of ultrasonication (minutes)
RP3 <sub>1</sub>	70	5
RP3 <sub>2</sub>	70	10
AV <sub>1</sub>	70	5
AV <sub>2</sub>	70	10

RP3 = Recycled pulp, AV = Avicel

**Table 2:** Treatment conditions for sonochemical-assisted maleic acid hydrolysis of Avicel and recycled pulp

Treatments	Maleic acid concentration (Mm)	Time of ultrasonication (minutes)	Power output of ultrasonicator (%)	Temperature of water-bath (°C)
1	50	6	60	15
3	100	6	60	35
5	50	9	60	35
7	100	9	60	15
9	50	6	90	35
11	100	6	90	15
13	50	9	90	15
15	100	9	90	35
Center-point	75	7.5	75	25

#### Preparation of Cellulose Nanocrystals

##### Deionized water

200 mg of Avicel and recycled pulp were weighed into 500 ml beakers. Deionized water (300 ml) was added to the lignocellulosic material. The resulting suspension was sonicated (at 15 °C and 35 °C) using a Vibra-Cell VCF1500 (1500W, 20 KHz, Sonics and Materials, Inc., Newton, CT) ultrasonicator probe at 70% power output for times as specified by treatment (Table 1). The appearance of a colloidal solution was a clear indication of the presence of cellulose nanocrystals. A suspension of cellulose nanocrystals was obtained by decanting the supernatant into new sample containers. Yields of cellulose nanocrystals from recycled pulp and Avicel were 2 and 5% dry weight of the starting material respectively. Samples of the supernatant

solution from each treated lignocellulosic materials were taken and characterized using a transmission electron microscopy.

#### Maleic acid

For maleic acid hydrolysis, 100 mg of Avicel was placed into 400 ml beakers and 300 ml of 50 mM and 100 mM maleic acid solutions (at 15 °C and 35 °C) respectively added as per the fraction factorial design (Table 2). The resulting suspension was centrifuged several times at 8,000 g and washed with distilled deionized water until the supernatant became turbid. This was done to remove the maleic acid. The resulting mixture was further sonicated until the supernatant becomes turbid. The suspension of cellulose nanocrystals was obtained by decanting the supernatant into new sample containers. This step produced an improvement in the yield of the cellulose nanocrystals (10% dry weight of the starting material). Aliquots of the supernatant solution from each treatment were used for the morphological characterization of cellulose nanocrystals using the transmission electron microscopy protocol described previously.

#### Characterization of Cellulose Nanocrystals

##### Transmission electron microscopy (TEM)

About 0.5 µl of each suspension of cellulose nanocrystals per treatment was loaded onto a 300-mesh carbon coated formvar copper grids (Electron Microscopy Sciences, Hatfield, PA, U.S.A.) using a Labnet micropipette. Water in the suspensions on the carbon coated grids was allowed to evaporate. Additional drop of each cellulose nanocrystals suspension was added onto their respective grids to increase the amount of cellulose particles and the process repeated. Cellulose nanocrystals coated grids were examined using JEOL 100CXII transmission electron microscope



at 80 kV. The dimensions of the imaged cellulose nanocrystals were determined by using the transmission electron microscope software.

## **Results and discussion**

### Scanning Electron Microscopic (SEM) characterization of Avicel and recycled pulp

The morphologies and dimensions of the two materials, Avicel and recycled pulp, determined by SEM analysis are shown in Figure 1. Avicel was irregular in shape with length ranging from 1 to 50  $\mu\text{m}$  and width from 1 to 20  $\mu\text{m}$ . In contrast, recycled pulp was cylindrical in shape with lengths ranging from 150 to 250  $\mu\text{m}$  and width from 20 to 50  $\mu\text{m}$ .

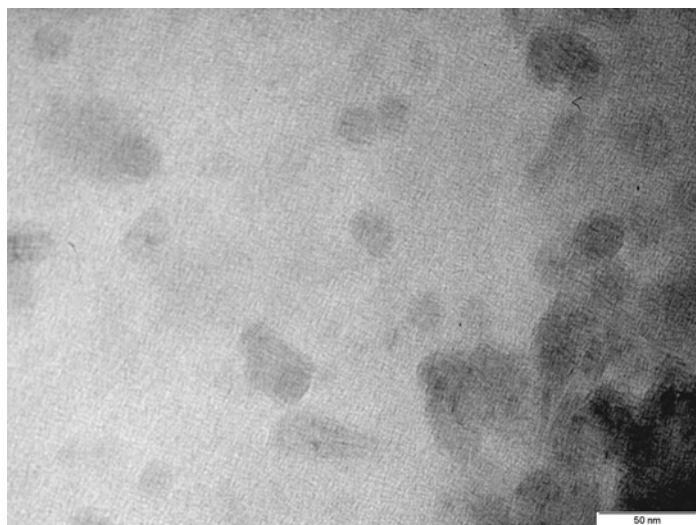
### X-ray diffraction of Avicel and recycled pulp

Crystallinity index, which is a measure of the crystalline cellulose content, was higher for Avicel (78%) than recycled pulp (63%) before treatment. After ultrasonic mediated maleic acid hydrolysis, the average CrI of both Avicel (81%) and recycled pulp (78%) residues increased. This was an indication of reduction and removal of amorphous component of cellulose in the starting lignocellulosic material. It is also a confirmation that amorphous domains of recycled pulp are more susceptible to hydrolysis than Avicel. Increase in the crystallinity of the recycled pulp was greater than for Avicel. The observed increase in crystallinity is an indication that a combination of a longer period of ultrasonication for maleic acid-mediated hydrolysis and/or higher concentration of maleic acid will improve the yield of cellulose nanocrystals.

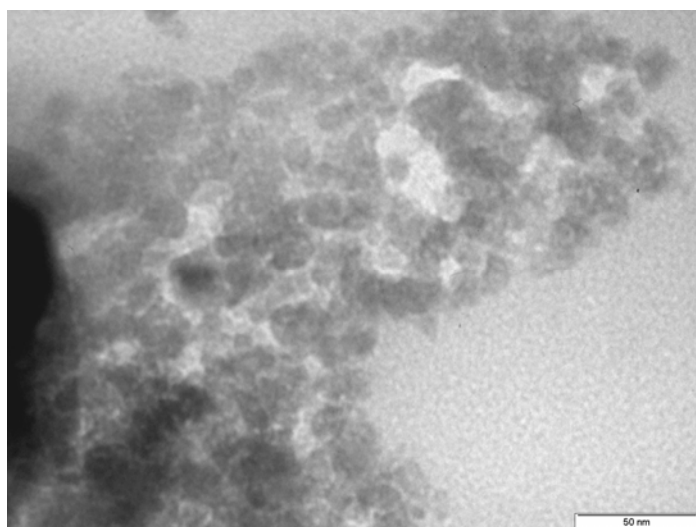
## Characterization of Cellulose Nanocrystals from Avicel and recycled pulp

### Deionized water

In deionized water, cellulose nanocrystals from Avicel and recycled pulp respectively exhibited different morphologies and dimensions (Figures 2 and 3). Transmission electron microscopic analysis showed that cellulose nanocrystals from Avicel were spherical with an average diameter (from 40 random particles) of  $21 \pm 5$  nm (minimum 15 nm and maximum 32 nm) (Figure 3). In solution, these nanoparticles tend to agglomerate and require extended period of sonication to reduce agglomeration. However, the size and shape remained fairly the same (Figure 3). In contrast, cellulose nanocrystals from recycled pulp were not distinctively spherical and their diameters averaged  $23 \pm 4$  nm (minimum 14 nm and maximum 32 nm, from 38 random particles) (Figure 4). The sizes and shapes of the nanoparticles are comparable to nanoparticles obtained from sono-chemical syntheses from Fe, Co and Fe-Co alloys (Suslick et al. 1991 and 1996). The similarity of sizes and shapes of cellulose nanocrystals to iron and iron alloys nanoparticles may be due to fragmentation of the materials from ultrasonication. Comparison of the diameters of cellulose nanocrystals using Student t-test showed no significant difference between their dimensions ( $t_{\text{critical}} = 1.70 > t_{0.05,33} = 1.43$ ).

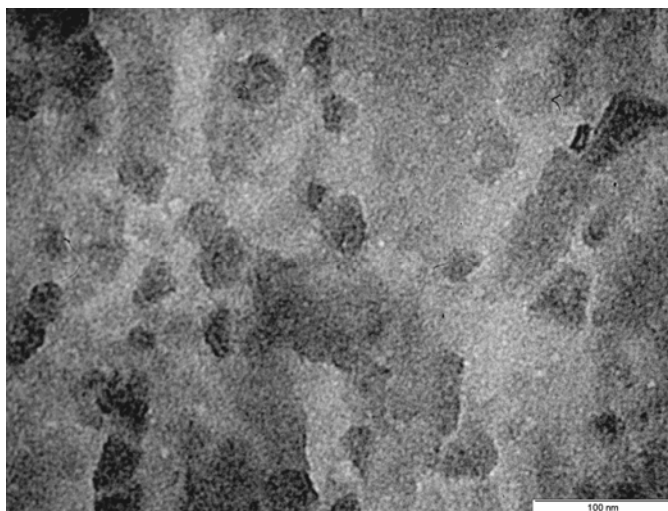


(a) AV-2

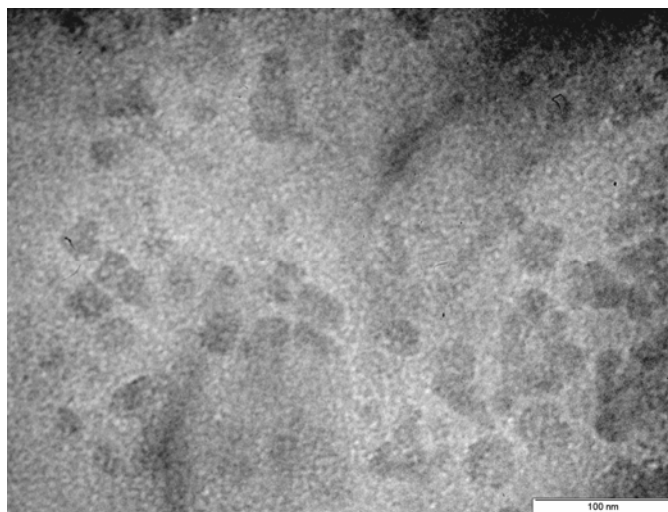


(b) AV-2

**Figure 3.** TEM images of cellulose nanocrystals produced from ultrasonication of Avicel (a) for 10 minutes and (b) for 5 minutes in water.

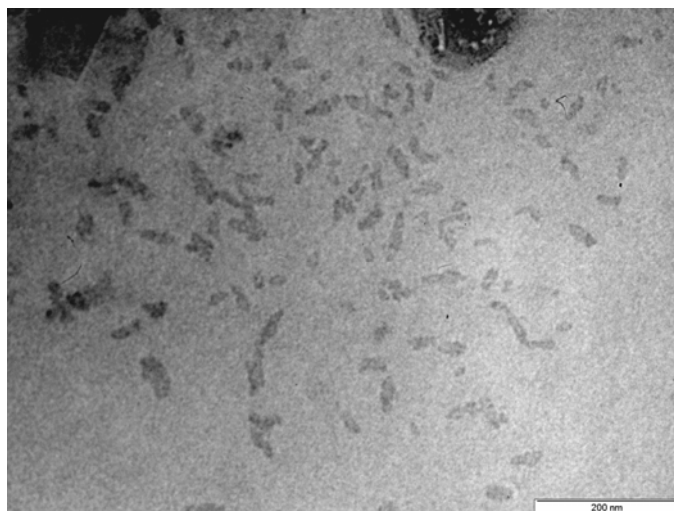


(a) RP3<sub>2</sub>



(b) RP3<sub>1</sub>

**Figure 4.** TEM images of cellulose nanocrystals produced from ultrasonication of recycled pulp (a) for 10 minutes and (b) for 5 minutes in water.



**Figure 5.** TEM image of cellulose nanocrystals produced from ultrasonication of Avicel in maleic acid (treatment 13).

#### Maleic acid

Of all the maleic acid treatments, only treatment 13 (Table 2) of Avicel produced cellulose nanocrystals. These were cylindrical with length and width of  $65 \pm 19$  nm and 15 nm respectively (Figure 5). The remaining treatments produced no cellulose nanocrystals. This observation may be due inadequate period and/or power output of the sonication step.

#### Conclusions

In deionized water, cellulose nanocrystals were produced from both recycled pulp and Avicel using ultrasonic mediated acid hydrolysis. Residues of both Avicel and recycled pulp showed increased crystallinity index after hydrolysis. This is an indication of reduced amorphous cellulose domains in both starting lignocellulosic materials. In deionized water, cellulose nanocrystals obtained from Avicel and recycled pulp respectively were of different morphologies. Cellulose nanocrystals from Avicel had an average diameter of  $21 \pm 5$  nm (minimum 15 nm and maximum 32 nm) from 40 particles. Recycled pulp, however, produced

cellulose nanocrystals that were not distinctively spherical and had average diameter of  $23 \pm 4$  nm (minimum 14 nm and maximum 32 nm). Only one treatment of Avicel with maleic acid produced cellulose nanocrystals. These nanocrystals were cylindrical in shape with larger dimensions, length of  $65 \pm 19$  nm and width 15 nm.

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## APPENDIX B

Paper II: Paul B. Filson and Benjamin E. Dawson-Andoh, Characterization of sugars from model and enzyme-mediated pulp hydrolyzates using high-performance liquid chromatography coupled to evaporative light scattering detection.

*Bioresource Technology 2008* (Under review)

### **Title:**

**Characterization of sugars from model and enzyme-mediated pulp hydrolyzates using high-performance liquid chromatography coupled to evaporative light scattering detection.**

### **Authors:**

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### **Abstract**

High performance liquid chromatography (HPLC) coupled to an evaporative light scattering detector was used to quantitatively determine glucose and cellobiose in hydrolyzates from the production of cellulose nanofillers from modified lignocellulosic materials. Prevail Carbohydrate ES 5 $\mu$  column proved more suitable for achieving the chromatographic separation of the model pulp hydrolyzate into its constituent sugars than the YMC-Pack Polyamine column. Linear calibration curves for the various sugars in the mixtures were developed. Glucose and cellobiose were clearly detectable in pulp hydrolyzates obtained from enzyme mediated hydrolysis of recycled pulp, pine and hardwood dissolving pulps. Finally, the amount of glucose in the pulp hydrolyzates was generally higher than cellobiose.

**Keywords:**

Recycled pulp, hardwood and pine dissolving pulps, sugars, glucose, cellobiose, endoglucanase, hydrolyzates.

**Main text:****1. Introduction**

Composite materials are made of two primary components: (1) matrix and (2) filler. Fillers contribute significantly to the strength properties of composites (Hull and Clyne, 1996). Traditionally, fillers such as carbon fiber, fiber glass, calcium carbonate, etc., are derived either from organic or inorganic sources. Recent studies, however, indicate that nanodimensional fillers (nanobiofillers) may be produced from renewable and sustainable sources such as lignocellulosic biomass. Lignocellulose consists of three main biopolymers: lignin, hemicelluloses and cellulose. The biopolymer cellulose is characterized by amorphous and crystalline regions.

The novel nanobiofillers, cellulose nanocrystals, are produced by first hydrolyzing the amorphous fraction of cellulose followed by physical fragmentation of the crystalline portion. Thus, cellulose nanocrystals are crystalline cellulose fragments with one dimension equal or less than 100 nanometers (Battista et al., 1953, Bondeson et al., 2006 and Wang et al., 2008).

When incorporated into compatible matrices, cellulose nanocrystals can significantly enhance the strength properties of the resulting composite [Wu et al., 2007; Samir et al., 2004; Kvien et al., 2007; Sugiyama et al., 2007 and Chakraborty et al., 2006]. The process of manufacturing cellulose nanocrystals from lignocelluloses generates a hydrolyzate which consists of mono- and oligosaccharides. These represent a potential feedstock for the production of biofuels and bioproducts. To accomplish this, these hydrolyzates need to be characterized.

To date, qualitative and quantitative determination of monosaccharides and other oligosaccharides in lignocellulosic biomass hydrolyzates are carried out using methods such as colorimetry (Lunder 1970 and Dubois et al., 1956), paper chromatography (Sunderwirth et al., 1966), gas chromatography (Magdolna et al., 1991 and Al-Hazmi and Stauffer 1986) and liquid chromatography (LC) (Momenbeik and Khorrasani 2006; Druzian et al., 2006 and Bernardez et al., 2004). Colorimetric determination of sugars is time consuming and gas chromatography

requires the laborious derivatization of sugars prior to elution and detection. Since the hydrolyzates are in the liquid state, the most commonly used characterization method has been high performance liquid chromatography (HPLC). This method is usually coupled to detectors such as electrochemical, UV-Visible (post-column derivatization) and refractive index.

In the last decade, evaporative light scattering detection (ELSD) has surfaced as one more important detector for HPLC. It can be used in situations where the analyte does not absorb in the Visible-Ultraviolet region. Unlike refractive index detector, it can be used in gradient elution mode. Evaporative light scattering detector is a very reliable and highly sensitive to low molecular weight non-volatile compounds including lipids (Perona et al., 1998; Hazotte et al., 2007) monosaccharides, and oligomers (Tao et al., 2006). However, the operating temperature of the column; pressure of the nebulizing gas, and tube temperature are very critical to the acquisition of a good response signal from the detector (Meyer 2006)

In this paper, we report the use of HPLC coupled to ELSD to optimize a method for determining the sugar content of enzymatic pulp hydrolyzate by-product from the production of cellulose nanofillers from recycled pulp, and pine and hardwood dissolving pulps.

## **2. Experimental**

### **2.1 Instruments, chemicals and mobile phases**

#### *2.1.1. Instruments*

The HPLC system used was a Waters 2695 separations module coupled to a Waters 2420 Evaporative Light Scattering (ELS) detector (Waters Corporation, Milford, Massachusetts, U.S.A.) with 45 psi pressurized ultra high purity nitrogen as nebulizing gas. The drift tube temperature was kept at 52 °C. This combined system was operated with Empower 2 software (Waters Corporation, Milford, Massachusetts, U.S.A.).

The column temperature was maintained at 35 °C. The injection volume and mobile phase flow rate used were 10 µl and 1 ml/min, respectively.

### *2.1.2. Hydrolyzate preparation*

Two different methods of heating for hydrolyzate preparation were tested. Conventional (Hybridization Incubator Combi-V12, FINEPCR, Yang-Chun, South Korea) and microwave-assisted heating at 50 °C for 60 minutes (MARS Xpress, CEM Corporation Matthews, North Carolina, U.S.A.) was followed. The microwave was programmed to ramp 50 °C in 20 minutes and hold at 50 °C for 60 minutes.

### *2.1.3. Chemicals*

Monosaccharides standards, glucose (99.5%), galactose (97.7%), xylose (99.5%), mannose (95.85%), arabinose (98.8%), rhamnose (99.5%) and cellobiose (99.2%), were purchased from TCI America, Portland, Washington, U.S.A. and used as received. They were dissolved in 4:1 acetonitrile-water to form model pulp hydrolyzates. HPLC grade acetonitrile (Fisher Scientific, New Jersey, U.S.A.) and deionised water were used. Recycled pulp was provided by American Fiber Resources (Fairmont, West Virginia). Recycled pulp boards were cut into pieces (approximately 25 × 25 mm) and milled in a Wiley mill fitted with a 120-mesh screen. The resulting fibers were used. Slurry of pine and hardwood dissolving pulps were provided by MeadWestvaco Corporation (Luke, Maryland). Specimen of the slurry of both pine and hardwood dissolving pulps were separately put in different beakers and placed in an oven and dried at 103°C for 24 hours to remove water. The resulting pine and dissolving pulps were later milled in a Wiley mill fitted with a 120-mesh screen. The resulting forms of the pine and hardwood pulps were used for the experiment.

### *2.1.4. Mobile phases*

Separation of model pulp hydrolyzates was carried out by both isocratic (4:1 acetonitrile-water) and gradient elution 4:1 acetonitrile-water to 7:3 acetonitrile-water were used.

### *2.1.5. Columns*

Two carbohydrate fractionating columns were used: (1) One was a Prevail Carbohydrate ES 5 $\mu$  (250 mm × 4.6 mm, 5 $\mu$ m) column (Grace Davison Discovery Sciences, Deerfield, Illinois,

U.S.A.) connected to All-Guard Cartridge System (Grace Davison Discovery Sciences, Deerfield, Illinois, U.S.A.) and (2) a YMC-Pack Polyamine II (250 mm × 4.6 mm, 5µm) column (YMC Company Limited, Kyoto, Japan) connected to pre-column (YMC Company Limited, Kyoto, Japan).

### *2.2 Preparation of standard sugar solution*

Standard stock sugar solutions of the various sugars were between 4000-4220 ppm of 4:1 acetonitrile-water in 50-ml volumetric flask. These stocks were serially diluted into different concentrations. Each standard stock sugar solution was filtered through a 10 ml syringe (Becton Dickinson and Company, New Jersey, U.S.A.) coupled to Fisher brand 0.45 µm PTFE filter (Millipore Corporation, Bedford, Massachusetts, U.S.A.). Five milliliters each of the standard stock sugar solution (highest concentration used) were combined in a 50-ml volumetric flask and stirred vigorously to ensure complete mixing. The resulting solution constituted our model pulp hydrolyzate. All the solutions were stored at 10 °C when not used. Two milliliters of each individual standard sugar solution of different concentrations and 2 ml of each of the mixture of the standard solutions were respectively analyzed using HPLC-ELSD.

### *2.3 Enzymatic mediated hydrolysis of dissolving and recycled pulps*

Two grams each of hardwood and pine dissolving pulp and recycled pulps were placed in separate 100 ml beakers. To each pulp was added 50 ml deionised water and the mixture was kept for 2 hours for the pulps to be softened. Then, 1 ml of endoglucanase enzyme (Celluclast 1.5 L, Novozyme AS, Franklinton, North Carolina, U.S.A.) was added to each mixture and stirred gently for 2 minutes. The resulting mixtures were each heated at 50°C for 60 minutes while stirring using either the microwave or the conventional protocol as described earlier in section 2.1.2. Immediately after heating, the mixtures were added to 50 ml of 95% ethanol (Fisher Scientific, Pittsburgh, Pennsylvania, U.S.A.) to stop the action of the endoglucanase. Each experiment was carried out in duplicate. Endoglucanase enzyme activity was assayed on carboxymethylcellulose using both microwave and conventional heating method. The hydrolyzates were filtered through a 0.45 µm PTFE membrane. Hydrolyzates from three pulps and carboxymethylcellulose were analyzed by comparing the retention times of the peaks of the

individual sugars to the chromatograms of the standard stock solution. Two controls were prepared, one without pulp and the other without the enzyme. Two ml aliquots of each mixture were used for HPLC-ELSD analysis.

#### *2.4 Statistical analysis*

The results obtained in this study were statistically analyzed by regression analysis using SigmaPlot version 10.0 (Systat Software, Inc., Chicago, Illinois, U.S.A.) to generate calibration curves.

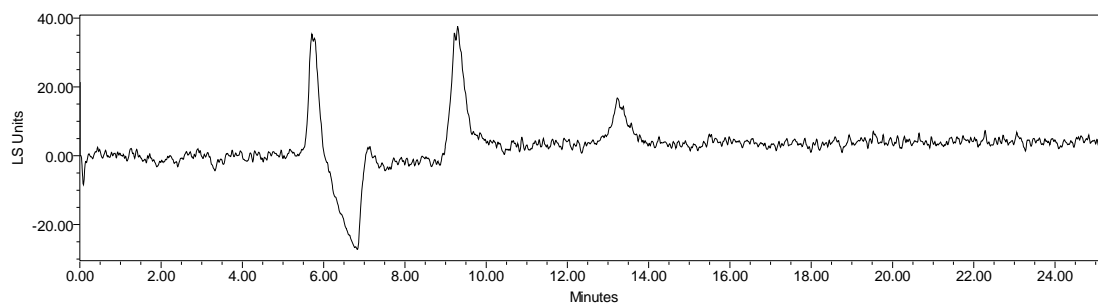
### **3. Results and discussion**

#### *3.1. Column comparison*

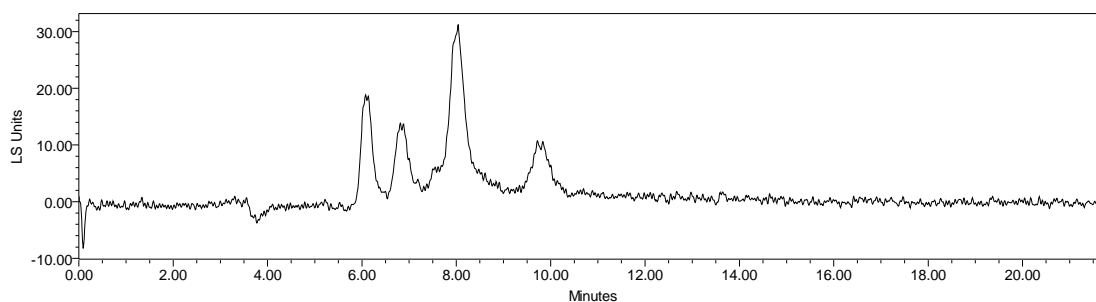
The focus of the work reported here was to develop and optimize a method for fractionating and quantifying the constituents of a model pulp hydrolyzate. The optimized method was then be used to characterize the constituents for unknown enzyme-mediated pulp hydrolyzate. Thus firstly, chromatograms of glucose, galactose, xylose, mannose, arabinose, rhamnose and cellobiose solution, were each developed separately using YMC-Pack Polyamine column at an optimized elution and detection conditions. This was done to establish the respective retention times for each sugar as shown in Table 1. The individual solutions showed different sensitivity at the same elution conditions. Mixtures of standard sugar solutions could not be resolved even after of elution and testing several mobile phase ratios and ELS detector settings. The number of peaks on the chromatograms was less than the expected seven for the seven sugars in the mixture (Figures 1 and 2).

The choice of YMC-Pack Polyamine column was made based upon the reported ability of this column to fractionate mixtures of monosaccharides (pentoses and hexoses) and oligosaccharides in other mixtures (Waters Corporation, 2006). Despite considerable time and effort spent testing other solvent systems and elution conditions to fractionate the model pulp hydrolyzate, no success can be reported. It was therefore inferred that, under the elution conditions used, YMC-Pack Polyamine column was not suitable for effective separation and detection of components of the model pulp hydrolyzate.

The individual sugar components of the standard sugar solution components were fractionated and identified on the Prevail Carbohydrate 5 $\mu$  column using solvent, elution conditions and ELS detector settings similar to that used previously for YMC-Pack Polyamine column (Section 3.1).



**Figure 1:** A typical chromatogram of combined sugar mixture using YMC-Pack Polyamine column Method: 70% acetonitrile: 30% water; column temp: 35°C; flow rate: 0.8 ml/min; gas pressure: 45 psi; drift tube temp: 52°C; nebulizer control: 60% and gain: 50.



**Figure 2:** A typical chromatogram of combined sugar mixture using YMC-Pack Polyamine column Method: 60% acetonitrile-40% water; column temp: 35°C; flow rate: 0.8 ml/min; gas pressure: 45 psi; drift tube temp: 52°C; nebulizer control: 60% and gain: 20.

Retention times of individual sugar solutions were longer than that obtained for the sugars on YMC-Pack Polyamine column (Table 1). Chromatogram of the combined sugar solutions (sugar mixtures) showed a good separation using gradient elution of the individual sugars better than that of YMC-Pack Polyamine column (Figures 1, 2 and 3). However, retention times of the individual sugars, in the mixture were not reproducible compared to the individual sugar solutions; this was ascribed to molecular interactions between the molecules of the various sugars in the mixture in relation to chemical groups of both the mobile phase and the column (Meyer 2006; Mikeš 1988) (Figure 3).

**Table 1:** Retention times,  $t_R$ , for selected carbohydrates using the YMC Pack and Prevail Carbohydrate ES columns. Elution conditions below table.

Carbohydrate	Column		
	YMC Pack Polyamine	Prevail Carbohydrate ES 5 $\mu$	
	Isocratic <sup>a</sup>	Isocratic <sup>b</sup>	Gradient <sup>c</sup>
Rhamnose (1)	5.7953	8.1114	7.5468
Arabinose (2)	5.4319	9.8728	9.2620
Xylose (3)	5.3909	10.5971	9.8520
Mannose (4)	5.9733	14.3721	13.2296
Galactose (5)	6.3200	15.4834	14.4027
Glucose (6)	6.3460	16.6959	15.3220
Cellobiose (7)	7.6345	27.9798	26.4035

**a:** Method: 60% acetonitrile-40% water; column temperature: 35°C; flow rate: 1.0 ml/min; gas pressure: 45 psi; drift tube temperature: 52°C; nebulizer control: 60% and gain: 10

**b:** Method: 80% acetonitrile-20% water; column temperature: 25°C; flow rate: 1.0 ml/min; gas pressure: 45 psi; drift tube temperature: 51°C; nebulizer control: 60% and gain: 50

**c:** Method: Eluent: 80% acetonitrile: 20% water to 70% acetonitrile: 30% water; column temp: 25°C; flow rate: 1.0 ml/min; gas pressure: 45 psi; drift tube temp: 52°C; nebulizer control: 60%; gain: 50 for mixture

**Table 2:** Calibration curves determined by external standard method

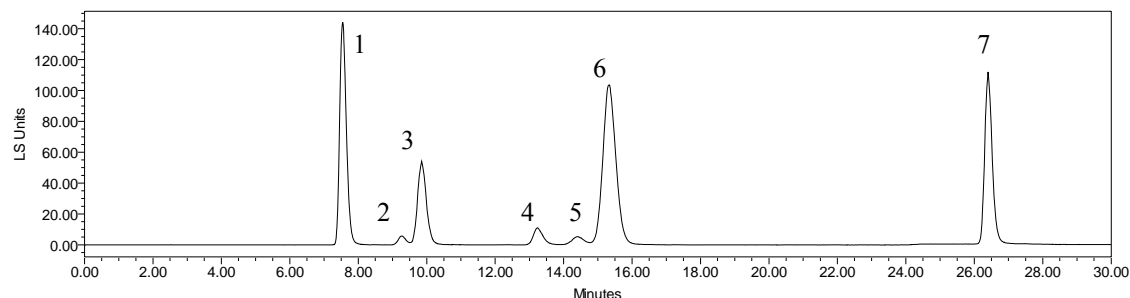
Carbohydrate	$n^a$	Slope (LSU/ppm)	Intercept (LSU)	$r^b$
Rhamnose (1)	7	0.0350	-19.548	0.9784
Arabinose (2)	7	0.0015	-0.8489	0.9855
Xylose (3)	7	0.0136	-7.9131	0.9752
Mannose (4)	6	0.0027	-1.7829	0.9663
Galactose (5)	6	0.0014	-0.8653	0.9612
Glucose (6)	7	0.0247	-14.179	0.9678
Cellobiose (7)	7	0.0284	-14.519	0.9772

<sup>a</sup> Number of different concentration points.

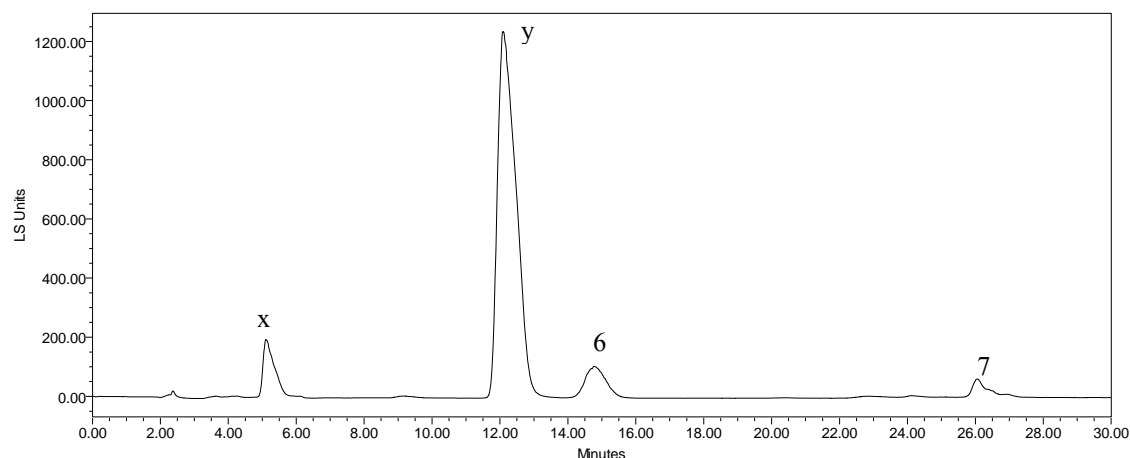
<sup>b</sup> Correlation coefficient

These results indicate that Prevail Carbohydrate ES 5 $\mu$  column is a suitable stationary phase for separating the sugars. Hence, this column was selected for the analysis of sugars in the hydrolyzates emanating from enzyme-mediated hydrolysis of dissolving hardwood (HP) and pine (PP) pulps and recycled (RP) pulp.





**Figure 3:** A typical chromatogram of combined carbohydrate mixture using Prevail Carbohydrate ES 5u column. Method: 80% acetonitrile: 20% water (20 minutes) to 70% acetonitrile: 30% water (30 minutes); column temp: 25°C; flow rate: 1.0 ml/min; gas pressure: 45 psi; drift tube temp: 52°C; nebulizer control: 60%; gain: 50 (1) rhamnose (4188 ppm), (2) arabinose (4064 ppm), (3) xylose (4084 ppm), (4) mannose (4220 ppm), (5) galactose (4072 ppm), (6) glucose (4148 ppm) and (7) cellobiose (4000 ppm)



**Figure 4:** A typical chromatogram of hydrolyzate of endoglucanase mediated hydrolysis of pulps. Method: 80% acetonitrile: 20% water to 70% acetonitrile: 30% water; column temp: 25°C; flow rate: 1.0 ml/min; gas pressure: 45 psi; drift tube temp: 52°C; nebulizer control: 60%; gain: 50; (x) and (y) are unknown from stabilizers in endoglucanase hydrolyzates.

### 3.2 Enzyme-mediated pulp hydrolyzates

Sugars in the hydrolyzates were identified by comparison of their retention times to that of standard sugars (Figures 3 and 4). These results showed the presence of glucose and cellobiose in the hydrolyzates from the enzyme-mediated hydrolysis of the pulps (Figure 4).

Two other compounds eluted at about  $t_R$  of 5.45 and 12.25 minutes as strong peaks (Figure 4). These two peaks were ascribed to compounds used as stabilizers in the endoglucanase enzyme preparation and were also in the chromatograms of the hydrolyzates from the control experiments. Calibration curves were developed for various concentration ranges of the sugars in the mixture to determine the amount of glucose and cellobiose in the enzymatic mediated pulp

hydrolyzates. Table 3 shows that glucose was produced in higher amount than cellobiose. This could be attributed to the efficacy of endoglucanase to hydrolyze the amorphous portion of cellulose to preferably glucose and some cellobiose.

**Table 3:** Composition of pulp hydrolyzates of endoglucanase mediated hydrolysis of recycled and dissolving pulps

	Conventional heating			Microwave heating		
Pulps	PP-1	RP-1	HP-1	PP-2	RP-2	HP-2
Glucose	8.2	5.9	6.9	6.1	12.3	5.5
Cellobiose	4.6	9.9	4.9	nd	2.9	nd

nd – not detectable

### *3.3 Composition of enzyme-mediated pulp hydrolyzates*

Table 3 shows both conventional and microwave heating lead to a generally higher amount of glucose than cellobiose in hydrolyzates from endoglucanase mediated hydrolysis of recycled pulp, hardwood and pine dissolving pulps. This could be ascribed to the efficacy of endoglucanase to mediate in the hydrolysis of glucose molecules from the amorphous regions of cellulose in pulps. However, there were no significant differences in the amount of glucose produced with the application of conventional and microwave heating. Recycled pulp with its low crystallinity (Wistara et al., 1991) was found to be more susceptible to hydrolysis. This was revealed in the highest amount of both glucose and cellobiose produced from it than that from pine and hardwood pulp (Table 3).

## **4. Conclusions**

The choice of stationary phase is very critical in the separation of sugars ( Biswas 2007). Prevail Carbohydrate ES 5 $\mu$  column coupled to an ELS detector proved more suitable than the YMC-Pack Polyamine II column for fractionation and identification of sugars from enzyme-mediated hydrolyzates of pulp. Glucose which was the major component of the enzyme hydrolyzates may serve as potential feedstock for the production of biofuels and biochemicals. Thus, production of the bio-nanofiller, cellulose nanocrystals, may be integrated with the production of biofuels and biochemicals.

## 5. Acknowledgement

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## APPENDIX C

Paper III: Enzymatic-mediated production of cellulose nanocrystals from recycled pulp.  
*Cellulose 2008* (Under review)

### **Enzymatic-mediated production of cellulose nanocrystals from recycled pulp**

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**Key words:** Cellulose nanocrystals, Recycled pulp, Endoglucanase, Microwave and conventional heating, Microscopy, Light scattering, design of experiment

#### **Abstract**

Endoglucanase was used to hydrolyze recycled pulp to produce cellulose nanocrystals aided with microwave and conventional heating. Design of experiment using factors including temperature of heating and time of heating, and nested with 1µl of endoglucanase per 1 mg recycled pulp produced cellulose nanocrystals at the highest yield at 50 °C for 60 minutes of microwave and conventional heating. Microwave heating at each treatment favorably yielded higher cellulose nanocrystals of initial weight of recycled pulp than the respective conventional heating. Transmission and scanning electron microscopic examination of suspension of cellulose nanocrystals showed sizes of cellulose nanocrystals (width 30-80 nm and length 100 nm to 1.8 µm) fell within the range (100 nm to 3.5 µm )for dynamic light scattering analysis. Light scattering was used to determine average zeta potential and molecular weight of cellulose nanocrystals in suspensions. X-ray diffraction of cellulose nanocrystals, recycled pulp and residue of recycled pulp showed gradual change in particle size.

#### **Introduction**

Cellulose nanocrystals represent a new emerging biological source of reinforcing biofillers. Various forms of lignocellulosic biomass are potential raw materials for the production of these new biofillers. These potential sources are also renewable, sustainable, abundant, and cheap. These new biofillers are of low density, high specific strength and modulus (Šturcova et al. 2005). Due to the presence of hydroxyl groups on the surfaces of cellulose nanocrystals, their surface are reactive making them suitable candidates as reinforcing material for the manufacture of composites (Favier et al. 1995 a, Grunert and Winter, 2002). Consequently, we have

witnessed the increasing application of cellulose nanocrystals as reinforcing materials for reinforced polymer nanocomposites. These nano-biofillers enhance the strength properties of resulting nanocomposites. To improve adhesion in nanocomposites containing hydrophobic matrices, hydroxyl groups on the surfaces of cellulose nanocrystals can be converted to hydrophobic groups through grafting and other methods (Grunert and Winter, 2002).

There are several starting materials that have been used to produce cellulose nanocrystals and they include microcrystalline cellulose [Araki et al. 1999, Bondeson et al. 2006], valonia (Grunert and Winter, 2002; Araki and Kuga, 2001), cotton (Montanari et al. 2005), wood pulp (Dong et al. 1996), tunicin (Favier et al. 1995 b) and sugar beet pulp (Dinandi et al. 1999). The commonly used method for the preparation of cellulose nanocrystals is mineral acid hydrolysis of cellulosic materials using sulfuric acid *ca* 64% (w/w). Cellulose nanofibers have also been produced from hardwood by treatment with 2,2,6,6-tetramethylpiperidine-1-oxyl radical in combination with sodium bromide (Saito et al. 2007). In the production of cellulose nanocrystals, acid hydrolysis of cellulosic materials is dependent on three factors: temperature, time and concentration of mineral acid (Bondeson et al. 2006). These factors affect the yield as well as the physical and mechanical properties of cellulose nanocrystals. The most commonly used protocol involves the hydrolysis of cellulosic materials with mineral acids at temperature range of 45 to 50°C depending on the time of hydrolysis and expected physical characteristics of the cellulose nanocrystals.

In the acid hydrolysis protocol, heating has traditionally been conventional heating. In conventional heating, energy is conveyed through convection, conduction and radiation (Venkatesh *et al.* 2004). However, rate of heating with conventional heating is slow compared to microwave heating. In microwave heating, electromagnetic energy is converted to thermal

energy through direct interaction of the incident radiation with the molecules of the target material (Venkatesh *et al.* 2004). Consequently, microwave heating is selective and specific in the processing of materials. Microwave may also reduce processing time and improve the quality of end products.

Current methods for producing cellulose nanocrystals are characterized by low yields (circa 20%). To commercialize production of cellulose nanocrystals, it is important to address the question of low yields associated with current processes. Cellulose consists of amorphous and crystalline regions. Since cellulose crystals are produced from the crystalline region, we hypothesized that the amorphous region can be selectively hydrolyzed using endoglucanase leaving the crystalline region. The latter is then fragmented into cellulose crystals using ultrasonic treatment. This reduces hydrolysis of the crystalline region to monosaccharides and thus enhance yield.

Cellulases is a composite of endoglucanases, exoglucanases and cellobiohydrolases. These enzymes act synergistically in the hydrolysis of cellulose (Rahkamo *et al.* 1997). Endoglucanase randomly attacks and hydrolyzes the amorphous region whilst exoglucanase attacks the cellulose polymer chain from either the reducing or non-reducing ends. Cellobiohydrolases hydrolyze cellobiose units to D-glucose.

Recycled pulp is largely cellulose with low lignin and hemicelluloses contents. Chemical and mechanical treatments of pulp in the recycling process increase the amorphous regions and reduce the cellulose chain length of cellulose molecules thereby decreasing crystallinity. By and large, recycle pulp makes poor quality paper. Consequently, several efforts have been made to improve the physical and chemical properties (Wistara and Young, 1999), crystallinity and surface properties (Wistara *et al.* 1999) of recycled fibers without much success.

Overall, recycled pulp may potentially be a viable raw material for the production of cellulose nanocrystals. In this paper, we report of the results of a study to produce cellulose nanocrystals from recycle pulp and also the effect of two modes of heating: conventional and microwave. The nanocrystals produced were characterized by a number of physical methods.

## **Experimental**

### *Materials*

Recycled pulp (1% lignin) was provided by American Fiber Resources (Fairmont, West Virginia, U.S.A.). Recycled pulp was produced from waste commercial wood pulp and used business papers. Endoglucanase, Celluclast 1.5 L FG was provided by AS Novozyme North America, Incorporated (Franklinton, North Carolina, U.S.A.) with density of 1.20 g/ml and a declared activity of 700 EGU/g. It was used as received. Sodium hydrogen phosphate buffer (1M, pH 6.8) was used. Deionized water was obtained using Corning Mega-pure (distilled water) and Barnstead E-pure purification systems (deionized water).

### *Heating methods*

Two methods of heating: (1) conventional (Hybridization Incubator Combi-V12, FINEPCR, Yang-Chun, South Korea) and (2) microwave (MARS Xpress, CEM Corporation, Matthews, North Carolina) were used. Microwave system was programmed to ramp to the desired temperature in 20 minutes and this was held constant the time periods indicated in Table 1. A ultrasonic bath, Branson 2510 (Branson Ultrasonics Corporation, Danbury, Connecticut, USA),



was used to sonicate suspensions of cellulose particles below 20 °C in order to further fragment presumably the unhydrolyzed large crystalline regions into cellulose nanocrystals.

### *Design of experiment*

Three primary factors: (1) hydrolysis temperature, (2) hydrolysis time and (3) endoglucanase enzyme concentration were explored. To reduce the number of treatments, a full factorial experimental design with two factors, hydrolysis temperature and hydrolysis time, were nested with two endoglucanase enzyme concentrations. Response factors evaluated were flow birefringence, appearance of turbidity in suspension, shape and size of cellulose particles.

Three hydrolysis temperatures: (1) 50°C (lower limit), (2) 60°C (upper limit) and (3) 55°C (center-point) and three hydrolysis times: (1) 45 minutes (lower limit), (2) 60 minutes (upper limit) and (3) 52 minutes (center-point) based upon literature review (Venkatesh *et al.* 2004; Rahkamo *et al.* 1997) were selected (Table 1). Two endoglucanase enzyme concentrations (1 ml/ 2 g and 1ml/g of pulp) per 200 mg of pulp respectively were used. A total of 60 treatments were generated by this experimental design.

**Table 1:** Overview of hydrolysis conditions for recycled pulp.

Heating method	Temperature (°C)	Time (minutes)
Conventional		
C-1	50	60
C-2	50	45
C-3	60	60
C-4	60	45
Center-point (CP-C)	55	52
Microwave		
M-1	50	60
M-2	50	45
M-3	60	60
M-4	60	45
Center-point (CP-M)	55	52

#### *Production of cellulose nanocrystals from recycled pulp*

To 200 mg of recycled pulp in a 125 ml beaker was added 25 ml of deionized water and phosphate buffer respectively and stirred with a magnetic stirrer for 2 hours to soften the pulp. 100 µl of endoglucanase enzyme was then added. Microwave heating and conventional heating were effected in a MARS Xpress laboratory microwave and hybridization incubator. Treatment solutions were placed in 75 ml vessels in MARS Xpress microwave or in a 80 ml vessel in a hybridization chamber and heated to the target temperature (Table 1) as described earlier. The experiment was later on repeated for treatments containing 200 µl of endoglucanase enzyme.

A control experiment for each treatment was carried out using deionized water and recycled pulp without endoglucanase enzyme. At the end of treatment procedure, 25 ml of 95% w/v ethanol was added to each resulting suspension and stirred vigorously with magnetic stir bars to terminate the action of endoglucanase.

The resulting suspensions were centrifuged at 12, 000 rpm at 10°C for 10 minutes using Sorvall RP-5B refrigerated superspeed centrifuge (DuPont Instruments, Chadds Ford, Pennsylvania, U.S.A.) and the supernate was decanted. Cellulose particles (mass) from each suspension were washed repeatedly with deionized water to remove endoglucanase enzyme from the solute until the supernate turned turbid. The turbidity was an indication of the presence and release of the cellulose nanocrystals. The turbid solutions were collected after 50 ml deionized water was added and sonicated in cold water bath for 2 minutes. Each suspension was allowed to stand for 30 minutes. The top turbid layer was decanted. This process was repeated several times until there was no indication of turbidity in each suspension. The cellulose residues from each treatment were further treated with half the initial amount of endoglucanase enzyme applied. The resulting suspensions were treated per above protocol until there was no turbidity. The total turbid solution collected for each treatment was filtered through 200 mesh sintered glass filter and evaporated at 50 °C in an oven until the volume of the suspension is reduced to about 25% of initial volume. The remaining suspension was freeze-dried and the cellulose nanocrystals vacuum dried to a constant dry mass. The yield was calculated as the percentage of the ratio of the dry mass of cellulose particles to the initial dry mass of recycled pulp.

### *Microscopic methods*

Images of flow birefringence of suspensions of the cellulose nanocrystals (0.1% w/v) between two cross polarizers were taken using Canon EOS digital camera (Figure 2). A droplet of 0.01% of each resulting cellulose nanocrystals suspension from each treatment was placed in a glass well slide and covered with a thin glass slide and imaged using Olympus bright field polarizing microscope at magnifications of 100X and 200X (Figure 3).

A droplet of cellulose nanocrystals suspension (0.001% w/v) was placed on a cleaned cut silicon surface and allowed to dry overnight. The silicon surfaces were imaged using Ultra-high resolution Hitachi S-4800 field emission scanning electron microscope (UHRFESEM) with operation accelerating voltage of 10 kV. The UHRFESEM was also used to image the initial recycling pulp fibers and the residues from each treatment. This was done to study cellulosic particles at each stage of endoglucanase enzyme mediated hydrolysis. Sample of cellulose nanocrystals in each suspension were imaged as performed by Filson and Dawson-Andoh (2008).



**Figure 1:** Scanning electron micrograph showing recycled pulp before cellulose hydrolysis.

About 0.5  $\mu\text{l}$  of each suspension of cellulose nanocrystals per treatment was loaded onto a 300-mesh carbon coated formvar copper grids (Electron Microscopy Sciences, Hatfield, PA, U.S.A.) using a Labnet micropipette. Water in the suspensions on the carbon coated grids was allowed to evaporate. Cellulose nanocrystals coated grids were examined using JEOL 100CXII transmission electron microscope at 80 kV. The dimensions of the imaged cellulose nanocrystals were determined by using the transmission electron microscope software.

### *Measurement of Dimension of cellulose nanocrystals using Light scattering*

Particle size distribution, average molecular weight and zeta potential of the various suspensions of cellulose nanocrystals from recycled pulp (0.01% w/v) from each treatment were determined using Nanotrak Ultra / Zetatrac instruments which operate on the theory of light scattering. Nanotrak Ultra / Zetatrac instruments determine particle size and polydispersity on the principle that particles in dispersion are in Brownian motion. These particles do scatter photons of light and there is an exchange of small energy between particles and photons. The diffusion coefficient of particles is measured and used to calculate the apparent hydrodynamic diameter of particles using Stokes-Einstein equation (Richardson, 2005). Zeta potential and average molecular weights of particles in suspension were determined based on the principle of electrophoretic and static light scattering respectively (Richardson, 2005).

### *X-ray diffraction studies of Cellulose Nanocrystals*

Samples of freeze-dried cellulose nanocrystals and residue from endoglucanase mediated hydrolysis of recycled pulp were first made into powder by grinding them using agate mortar and pestle. The resulting powders of cellulose nanocrystals, residue of recycled and initial recycled pulp were respectively placed separately on quartz with a rectangular depression with dimensions 1 mm X 20 mm X 15 mm. Ethanol (95%) was added to samples on quartz glass and allowed to evaporate. The sample was later pressed hard to flatten the surface to flush the plane of quartz before mounting on a stage to record X-ray diffraction patterns. Wide angle X-ray diffraction patterns of each sample of cellulosic materials were recorded using Rigaku Geiger

Flex D-Max 1000 diffractometer and fiber attachment using Ni-filter Cu K $\alpha$  radiation, generated with a rotating anode generator operating at 50 kV and 120 mA. The sample was exposed at an angle of incidence ( $\theta$ ) varied from 10° to 50° by steps of 0.06°.

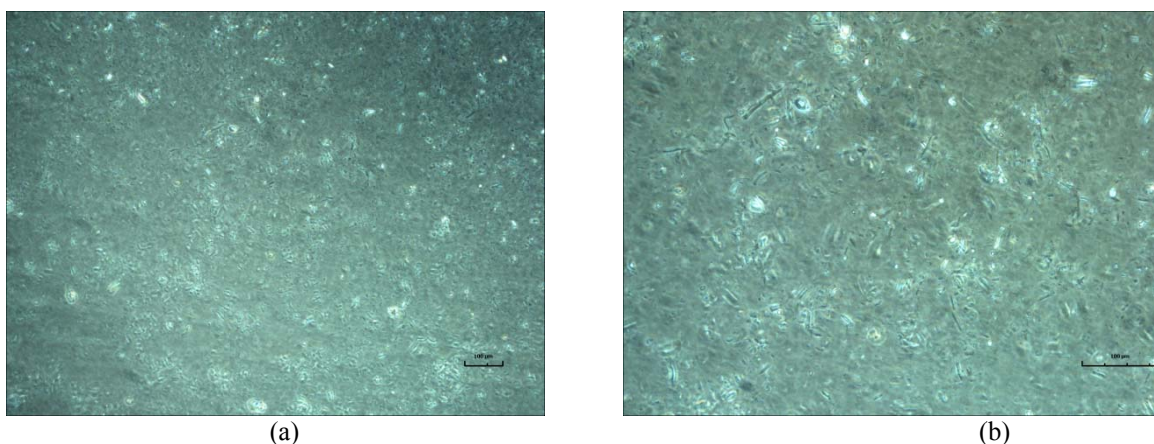
## **Results and discussion**

### *Particle size distribution of cellulose nanocrystals*

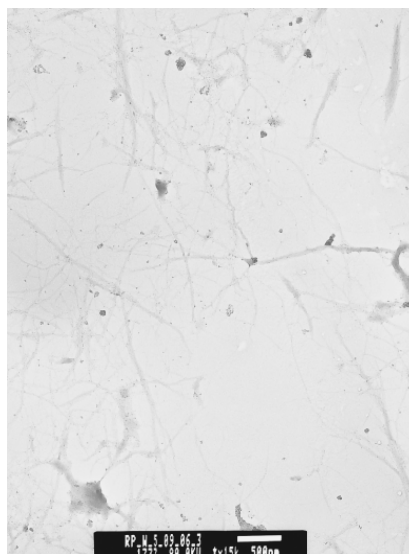
The presence of cellulose nanocrystals after each treatment was indicated by the presence of birefringence in the suspensions. This is due to polarization of plane light by nematic orientation of the cellulose nanocrystals. Birefringence of the cellulose nanocrystals suspension was imaged by first shaking for a short time and placing it between two polarizing films in a dark box (Figure 2). (Figure 3) Transmission and scanning electron micrographs (Figures 4 and 5) show that, cellulose nanocrystals have width between 30-80 nm and length between 100 nm to 1.8  $\mu$ m. However, the transmission electron microscopy images a small area of surface on substrate with the particles. Consequently, scanning electron microscopy was further used to image a larger surface area on the substrate to give a good report of the sizes of the particles in the suspensions. The sizes and shapes of the cellulose nanocrystals imaged using both TEM and SEM were in good agreement.



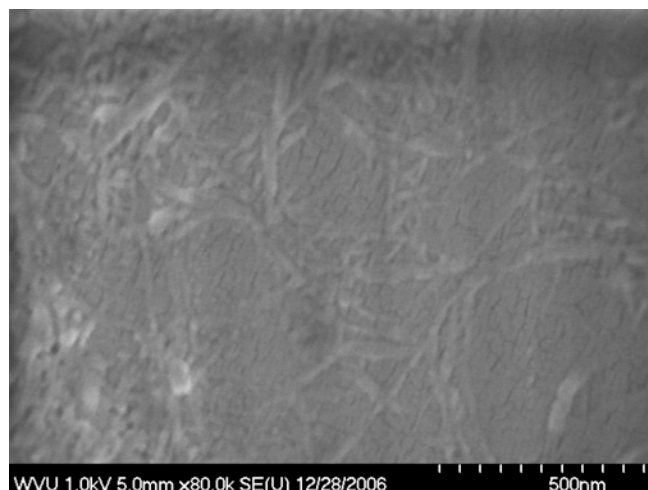
**Figure 2:** Flow birefringence of cellulose nanocrystals from recycled pulp between two cross polarizing films. (Container is 20 mm in diameter).



**Figure 3:** Cellulose nanocrystals seen under polarizing microscope at (a) 100X and (b) 200X. Cellulose nanocrystals agglomerate.



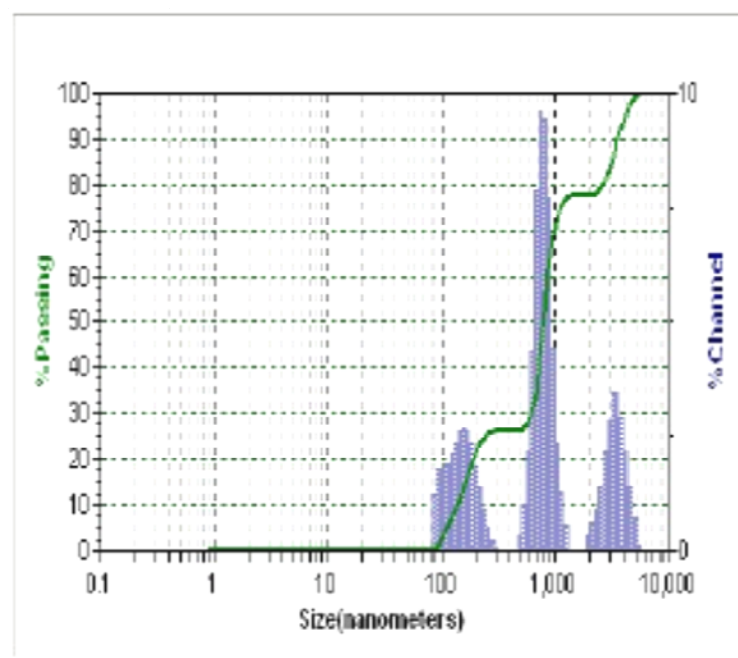
**Figure 4:** TEM images of cellulose nanocrystals from recycled pulp ( (Scale bar = 500 nm).



**Figure 5:** SEM images of cellulose nanocrystals from recycled pulp (Scale bar = 500 nm).

Results of dynamic light scattering studies on the sizes and distribution of cellulose nanocrystals in suspensions were compared with that of electron microscopy. The volume of cellulose nanocrystals suspension used for particle size and size distribution is large enough and give a better representation of the total volume of the suspensions than that used for the electron microscopy study. The average length of cellulose nanocrystals ranged from about 100 nm to 3.5  $\mu\text{m}$  (Figure 6). The cellulose nanocrystals size distribution exhibited a trimodal frequency distribution with average lengths of 154.9, 820 and 3540 nm for each peak. This was comparable to that obtained using the electron microscope. Finally, the average molecular weight of the cellulose nanocrystals was determined to be  $1.65 \times 10^{11}$  daltons. The unusually high average molecular weight can partly be ascribed to aggregation of the cellulose nanocrystals.





**Figure 6:** Particle size distribution of cellulose nanocrystals from recycled pulp

#### *Crystallinity of cellulose nanocrystal, recycled pulp and residue*

X-ray diffraction patterns show increase in the crystalline domains in the recycled pulp (residue) as recycled pulp (starting) undergo endoglucanase mediated hydrolysis (Figure 7) with the decrease in the amorphous region. This is evidenced by the presence of stronger diffraction peaks at  $2\theta = 16.8^\circ$  and  $22.6^\circ$  as compared to weak or no reflection at. Diffraction peaks at  $2\theta = 16.8^\circ$  and  $22.6^\circ$  are ascribed to cellulose I (native cellulose) and  $2\theta = 18^\circ$  is amorphous. This suggests that endoglucanase mediated hydrolysis of recycled pulp does not change polymorphism of cellulose I in the residue as well as cellulose nanocrystals produced. However, the average crystallite sizes show progressive increase in size from cellulose nanocrystals through recycled pulp (starting) to recycled pulp (residue) using Scherrer's equation below. Furthermore, X-ray diffraction patterns (Figure 7) show that cellulose nanocrystals have broader

X-ray diffraction peak which further supports that they have smaller particle size than both recycled pulp (initial) and recycled pulp (residue)( Suryanarayana and Norton 1998).

Scherrer's equation:

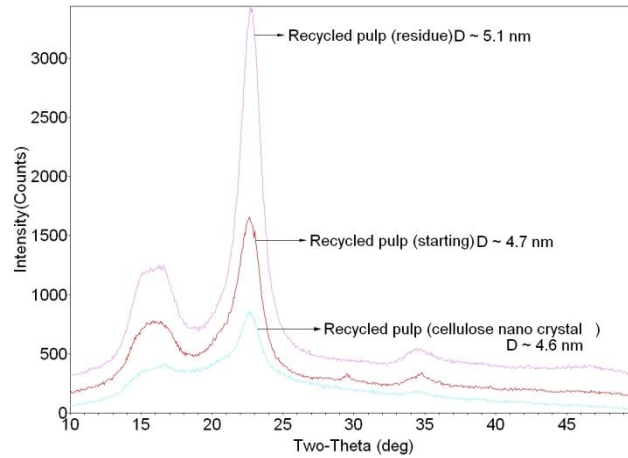
$$D = \frac{k\lambda}{B \cos \theta}$$

where D = Average crystallite (particle) size perpendicular to direction of X-ray

$\theta$  = Bragg's angle

$\lambda$  = wavelength of X-rays used

B = full width at half maximum of diffraction peak



**Figure 7:** X-ray diffraction patterns of cellulose nanocrystals, recycled pulp (initial) and recycled pulp (residue)

### *Zeta potential and stability of cellulose nanocrystals suspensions*

Stability of suspensions of nanoparticles is critical in the preparation of nanocomposites and it is derived from the absolute magnitude of zeta potential of suspensions. Average zeta potential was determined to be -31.37 mV which reflects the favorable stability of the suspensions for extended period of time. Voltage recorded is an indication that suspension of cellulose

nanocrystals from recycled pulp was fairly stable for a period of time (Correia et al. 2004). This was exhibited from the stability of suspensions of cellulose nanocrystals for over a month before they aggregate and settle at the bottom of containers (Figure 8).



**Figure 8:** Suspensions of cellulose nanocrystals showing gradual aggregation of cellulose nanocrystals with time.

#### *Effect of conventional and microwave heating on the yield cellulose nanocrystals*

Table 2 shows various treatments of recycled pulp with endoglucanase enzyme at different temperatures (50 °C, 55 °C and 60 °C) in different media: (1) (water and endoglucanase), (2) phosphate buffer and endoglucanase), and (3) water only and also response factors for each treatment condition for preparation of cellulose nanocrystals. Yield of cellulose nanocrystals in water and endoglucanase were higher at all modes of heating (Table 1). Additionally, endoglucanase treatments at 50 °C in water at both heating modes gave the highest yield of cellulose nanocrystals (29 % for conventional and 38.2% for microwave heating). This may therefore represent the optimum temperature for endoglucanase hydrolysis of the amorphous regions of recycled pulp in the reaction media. Comparatively, yield of cellulose nanocrystals were higher for microwave mode of heating. This may be a reflection of the relative efficiency and specificity of microwave in heating materials (Venkatesh *et al.* 2004)

**Table 2:** Experimental results for properties and yield of cellulose nanocrystals from recycled pulp

Conventional heating	Factor responses			
	Characteristics of suspension	Flow birefringence	Shape of particles	Yield (%)
C-1-E	Turbid	Yes	Cylindrical	10.7
C-1-B	Clear	No	-	-
C-1-W	Turbid	Yes	Cylindrical	7.4
C-2-E	Turbid	Yes	Cylindrical	29.0
C-2-B	Turbid	Partial	Cylindrical	25.7
C-2-W	Turbid	Yes	Cylindrical	7.1
C-3-E	Turbid	Yes	Cylindrical	15.0
C-3-B	Clear	Partial	Cylindrical	21.0
C-3-W	Turbid	Yes	Cylindrical	3.7
C-4-E	Turbid	Yes	Cylindrical	3.4
C-4-B	Turbid	Partial	Cylindrical	18.0
C-4-W	Turbid	Yes	Cylindrical	1.5
CC-E	Turbid	Yes	Cylindrical	16.0
CC-B	Clear	Partial	Cylindrical	11.0
CC-W	Turbid	Yes	Cylindrical	4.9
Microwave heating				
M-1-E	Turbid	Yes	Cylindrical	34.9
M-1-B	Clear	Partial	Cylindrical	10.9
M-1-W	Turbid	Yes	Cylindrical	12.8
M-2-E	Turbid	Yes	Cylindrical	38.2
M-2-B	Turbid	Yes	Cylindrical	26.4
M-2-W	Turbid	Yes	Cylindrical	4.9
M-3-E	Turbid	Yes	Cylindrical	22.9
M-3-B	Clear	Partial	Cylindrical	15.9
M-3-W	Turbid	Yes	Cylindrical	6.2
M-4-E	Turbid	Yes	Cylindrical	20.5
M-4-B	Turbid	Yes	Cylindrical	15.0
M-4-W	Turbid	Yes	Cylindrical	6.6
MC-E	Turbid	Yes	Cylindrical	18.2
MC-B	Clear	Partial	Cylindrical	13.0
MC-W	Turbid	Yes	Cylindrical	6.0

C = convectional heating; M= microwave heating; CC = conventional heating at center-point temperature; MC = microwave heating at center-point temperature; E = Enzyme in deionized water; B =Enzyme in sodium phosphate buffer and W = water only.

## **Conclusions**

The treatment of recycled pulp with endoglucanase enzyme at all the experimental conditions of temperature, media and pH produced cellulose nanocrystals at different yields. Cellulose nanocrystals yield were higher in water and endoglucanase at both modes of heating. For both modes of heating, endoglucanase enzyme treatment in water gave the highest cellulose nanocrystals yield.

In all treatments, presence of cellulose nanocrystals was confirmed by flow birefringence. Transmission electron microscopy showed that cellulose nanocrystals had width between 30-80 nm and a length of 100 nm to 1.80  $\mu\text{m}$ . Dynamic light scattering studies showed that cellulose nanocrystals exhibited a length of 100 nm to 3.5  $\mu\text{m}$ . Size distribution of cellulose nanocrystals showed a trimodal frequency distribution with an average length of 154.9, 820 and 3540 nm for each peak. X-ray diffraction indicated an increase in crystallinity as the amorphous domains in the recycled pulp is reduced by endogluacnase enzyme hydrolysis.

Cellulose nanocrystals had an average zeta potential of -31.37 mV, an indication of a favorable stable cellulose nanocrystals for extended period of time. The stability of suspensions of cellulose nanocrystals in deionised water suggests their suitability as nanofillers in the making of cellulose nanocrystals reinforced polymer nanocomposites. The success of this study gives a potential green method for the production of cellulose nanocrystals using endoglucanase.

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Wistara N., Zhang X., and Young R. A. 1999. Properties and treatments of pulp from recycled paper. Part II. Surface properties and crystallinity of fibers and fines. *Cellulose* 6, 325-348

## CURRICULUM VITAE

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#### SUMMARY

- Expertise with FTIR-PAS/ATR, Raman, UV, HPLC-UV/ELSD/RI, TLC, Flash and column chromatography, NMR, MS and miscellaneous chemical analytical methods.
- Expertise in synthesis of cellulose nanocrystals/cellulose nanocrystals reinforced-polymer nanocomposites and characterization using light scattering, SEM, TEM, AFM, TGA, DSC and XRD.
- Working experience with FT-NIR to discriminate materials
- Expertise in isolation and structural elucidation of bioactive compounds from plants
- Synthesis and characterization of benzocrown ethers.

#### EDUCATION

West Virginia University	Forest Resource Sciences	Ph.D.	Expected Dec. 2008
University of Ghana	Organic Chemistry	M.Phil.	2003
Tufts University	Chemistry	Visiting Student	2000/2001
University of Ghana	Chemistry	B.Sc.	1998

**PhD Dissertation:** Novel methods of production of cellulose nanocrystals from pulp and cellulose nanocrystals reinforced nanocomposites. Advisor: Dr. Benjamin E. Dawson-Andoh

- Endoglucanase mediated production of cellulose nanocrystals from lignocellulosics
- Application of ultrasonication to produce cellulose nanocrystals
- Characterization of nanocrystals using light scattering, SEM, TEM and AFM
- Thermal analysis and mechanical properties determination of biomaterials

**Master's Thesis:** Determination of structure and antiplasmodial activity of compounds from *Teclea verdoorniana* Exell and Mendonca and *Rothmania longiflora* Salisb. Advisor: Dr. William A. Asomaning (University of Ghana, Ghana)

- Isolation and structural elucidation of bioactive compounds from plants
- Phytochemical screening of plant extracts
- High performance liquid chromatography-ultraviolet/light scattering/refractive index and thin-layered chromatographic techniques in the analysis of compounds

#### EXPERIENCE

**West Virginia University-** Graduate Research Assistant (Fall 2004 to date)

- Expertise with XRD, SEM, TEM, AFM, FTIR-PAS/ATR, Raman, UV, HPLC-UV/ELS/RI, TLC, NMR, MS and miscellaneous chemical analytical methods
- Expertise in synthesis of cellulose nanocrystals and cellulose nanocrystals reinforced polymer nanocomposites
- Thermal analysis and mechanical properties determination of cellulose nanocrystals and cellulose nanocrystals reinforced polymer nanocomposites
- Extensive experience with design of experimental methods

**West Virginia University-** Teaching Assistant/Mentor (Fall 2005- Spring 2007)



- Mentored undergraduate students during Summer 2005 and 2006 (under Summer Undergraduate Research Experience Program) to carry out independent research.
- Taught undergraduate class on wood anatomy and wood physics

#### **University of Ghana -Teaching assistant/Laboratory Coordinator (Fall 2001 - Fall 2004)**

- Put together chemistry laboratory work sheet for freshmen and sophomore students and organized laboratory schedule.
- Taught organic chemistry and general chemistry for undergraduate students
- Graded chemistry laboratory worksheets for freshmen and sophomore students

#### **PRESENTATIONS**

- U. Salma, L. Matuana, B. Dawson-Andoh, P. Filson and P. Heiden- Preparation of Core-Shell Nanoparticles for controlled release of wood preservatives. International Conference on Natural Polymers, Kottayam, Kerala, India, November, 2007
- U. Salma, L. Matuana, B. Dawson-Andoh, P. Filson and P. Heiden - Preparation of Core-Shell Nanoparticles for Controlled Release of Biocides. XX Sociedad Polimerica de Mexico (SPM) National Congress of Polymers, Guanaguato, Mexico, November 19-21, 2007
- P. B. Filson and B. E. Dawson-Andoh, Enzymatic-assisted production of cellulose nanocrystals from lignocelluloses materials, 39th Central Regional American Chemical Society Meeting, Covington, KY, May 20-23, 2007.
- P. B. Filson, B. E. Dawson-Andoh, D. Schwegler-Berry and William E. Wallace, Enzymatic-assisted production of cellulose nanocrystals from lignocelluloses materials, 2<sup>nd</sup> Science Technology Research (STaR), Morgantown, WV, September 17-18, 2007
- O. P. Kryatova, P. Filson E. V. Rybak-Akimova. "Cyclidene-based ditopic receptors for  $\alpha,\omega$ -ammonium salts". Abstracts of Papers, 224th ACS National Meeting, Boston, MA, United States, August 18-22, 2002 (2002), INOR-162.

#### **PUBLICATIONS**

- Paul B. Filson, Benjamin E. Dawson-Andoh and Diane Schwegler-Berry. "Enzymatic-mediated production of cellulose nanocrystals from recycled pulp". *Cellulose 2008* (Under review)
- Paul B. Filson, Benjamin E. Dawson-Andoh, "Characterization of sugars from model and enzyme-mediated pulp hydrolyzates by high-performance liquid chromatography using Evaporative Light Scattering detector," 2008, (In review)
- Paul B. Filson, Benjamin E. Dawson-Andoh, "Sonochemical preparation of cellulose nanocrystals from lignocellulosic materials", *Bioresource Technology*. 2008 (In print)
- P. Filson, B. Dawson-Andoh and L. Matuana, "Colorimetric and Vibrational Spectroscopic Characterization of Weathered Surfaces of Wood and Rigid Polyvinyl Chloride-Wood Flour Composite Lumber", *Wood Science and Technology*, 2008.(In print)
- D. Obeng-Ofori, I. E. Aidoo, R. K. Akuamoah, and P. B. Filson, "Evaluation of Leaf Extracts of the Siam Weed *Chromolaena odorata* (L.) and Mahoghany Tree *Khaya senegalensis* (Desr.) Against the Maize Weevil *Sitophilus zeamais* (Mot.)". *Agricultural and Food Science Journal of Ghana* July 2002, Vol. 1, 33-36.

#### **TRAINING/RESEARCH PROGRAMS**

- Center for Nanoscale Materials at Argonne National Laboratory, Argonne, IL June 7-30, 2008

- National School on Neutron and X-ray Scattering at Argonne National Lab, Argonne, IL August 14-28, 2005
- Training on FT-NIR at Bruker Optics, Billerica, MA, March 27-31, 2005

## **PROJECTS**

- Synthesis and characterization of benzocrown ethers
- Spectroscopic and colorimetric study of weathering of wood plastic composites
- Rapid identification of wood-decay fungi using Infrared spectroscopic methods
- Controlled release of biocides from core shells of polymer nanoparticles

## **AFFILIATIONS**

Graduate Student Association (President Sept. 2004 to May 2007); American Chemical Society (2005– to date); Neutron Scattering Society of America (2005– to date); Society of Wood Science and Technology (2006 – to date) and Gamma Sigma Delta (2006– to date)

## **COMPUTER AND OTHER SKILLS**

- Extensive literature search experience using electronic databases such as Scifinder Scholar, Web of Science and others
- Proficient in Excel, MS Word, SigmaPlot, MS PowerPoint, ChemDraw, ACD-NMR LabManager, Unscrambler and others
- Good writing and communication skills
- Conversational French and Hausa
- A good team player

## **REFERENCES**

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